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Genetics of Olfactory Behavior in *Drosophila melanogaster*.

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SUMMARY

We have used a behavioral genetic approach to identify six X-linked loci which specify olfaction in *Drosophila melanogaster*. Mutations in five of these genes lead to partial anosmias affecting responses either to aldehydes (*olfA*, *olfB*, *olfE* and *olfF*) or to acetate esters (*olfC*). Only one of the mutants obtained in our screening (*olfD*) resulted in a insensitivity to several different odorants. *olfA*, *olfE* and *olfC* map close together in a small region of the chromosome between 7C and 7D. The alleles at the *olfC* locus fall into two phenotypic classes according to their responses to different acetate esters. The two groups of *olfC* alleles interact *in-trans*.

INTRODUCTION

Drosophila melanogaster possesses a well developed sense of smell and is capable of discriminative behavior towards a variety of odorants (Rodrigues, 1980) One approach to understand the mechanisms of olfaction is to study mutants of Drosophila defective in olfactory behavior. A number of investigators have analyzed olfactory mutants of Drosophila employing different test paradigms. (Kikuchi et al, 1973; Fuyama, 1976; 1978; Rodrigues and Siddiqi, 1979; Becker, 1983; Helfand and Carlson, 1989, McKenna et al,1989, Woodward et al, 1989; Lilly and Carlson, 1990). The olfactory mutants reported so far affect the fly's response to one or more classes of chemicals. Some of the loci affect olfaction specifically; others also impair diverse sensory modalities such as taste or vision (McKenna et al, 1989; Lilly and Carlson, 1990).

A behavioral defect could result from a lesion at any point of the olfactory pathway ranging from stimulus recognition to transduction, coding and decoding of sensory signals and finally the motor output. The peripheral detectors for olfactory stimuli are the neurons in the sensilla of the antenna. Electrophysiological recordings from single units show that the neurons innervating the sensilla basiconica exhibit characteristic response spectra, each neuron responding to a subset of odorants. The antennal neurons connect to the first relay station in the glomeruli of the antennal lobes (Stocker et al, 1983; Siddiqi, 1987). It is therefore possible to delimit the defective step to either peripheral or central events.

In 1978, we described a set of olfactory genes on the X chromosome which cause specific anosmias (Rodrigues and Siddiqi, 1978). A detailed account of these mutants remained unpublished, although some of them have been used in behavioral experiments by other workers (Venard, 1980; Tompkins and Hall, 1981; Tompkins at al, 1983; Venard and Pichon, 1984; Gailey et al, 1986; Lilly and Carlson, 1990). In this paper, we describe the properties of six X-linked genes. Four of these were briefly reported earlier (Rodrigues and Siddiqi, 1978; Rodrigues, 1980). Two additional genes were identified in later experiments.

MATERIALS AND METHODS

Stocks.

The Canton Special strain (CS) was obtained from S. Benzer, CALTECH, California. It was isogenized and used as the wildtype strain in all subsequent experiments The sources and genotypes of the deficiency and duplication strains are given in Table 1. The balancer chromosomes- FM4, FM6 and FM7; the multiply marked strains - *y cv v f car* and *cm ct sn* and markers are described in Lindsley and Grell,(1972). These stocks were obtained from the Mid-Western University Stock Center at Bowling Green.

Chemicals.

Benzaldehyde and iso-amyl acetate were obtained from Sigma chemicals U.S.A.; Propionic acid, acetic acid and liquid paraffin (Art 7162) were obtained from E. Merck, Darmstadt, W.Germany. n-butanol, ethanol and cyclohexanone were from Sarabhai Chemicals, Baroda, India. Aliquots of the stock solutions were stored under nitrogen in light tight bottles.

Tests of Olfactory Behavior.

Larval assay. This test was designed by Aceves-Pina and Quinn, 1979 and has been recently described in detail by Monte et al(1989). Third instar larvae collected from several bottles are pooled, washed and placed in *Drosophila* Ringer. A batch of approximately 100 larvae are gently blotted dry and placed in the center of a Petri plate containing 1% agar. 25ul of test chemical appropriately diluted are added to a filter paper disc (diam 1cm) placed at one end of the plate. An equal volume of the diluent is placed at the opposite end. When the lid is covered, an odor gradient is rapidly formed and the larvae move to the smell side of the plate. After five minutes, the larvae on each half of the plate are counted. The Response Index (R.I.) is calculated from the difference in the number of larvae on the smell and control halves of the plate divided by the total count. An R.I. of +1 indicates total attraction and -1 total repulsion. Since larvae exhibit strong responses to light, the test is carried out in a room protected from external lighting and illuminated by ceiling lights placed directly above the work area. The test is repeated with the plates oriented in the four different directions. A single R.I. is computed from the average of the four runs. In all experiments where mutant phenotypes are being measured, wildtype responses are measured in parallel.

Olfactometry of the imago

In principle two different types of olfactometric tests can be employed. In the "trap test", the number of flies entering a compartment containing a source of odor are counted (Barrows, 1907). In a binary choice test the flies are given a choice between two directions, one of which bears an odor cue (Begg and Hogben, 1943)

1.Design of the olfactometer

We have employed an olfactometer based on the designs of Fuyama (1976) and Rodrigues and Siddiqi(1978). The olfactometer is a simple Y-maze made of glass (Fig 1A). The junctions of the Y are joined to the arms by ground glass joints. The tapered ends of the arms are connected by polythene tubing to glass bottles containing 100ml solutions of the odorant and the diluent respectively. Air is drawn from the stem of the Y-tube by an aquarium suction pump at the rate of 0.5 liter/min. This causes a symmetric inflow of smell and control air streams through the arms of the maze. The rate of bubbling in the two gas bottles is used to equalize the flow rate through the maze using pinch corks.

2.Preparation of flies

Two to six days old flies are used in all experiments. When the response to attractants is being measured, the flies must be starved for 18hrs prior to the test. Before starvation, they are fed with sucrose by placing them into bottles containing filter paper soaked in 1% sucrose. They are transferred into bottles containing filter paper moistened in distilled water and left at 22^{o}C for 18hrs.

Starvation is not required when measuring responses to repellents. In this case, flies are placed in bottles with moistened filter paper for one hour prior to the test when they preen themselves clean of food material carried from the culture bottles.

3.Olfactometry

The olfactometer can be used under two different conditions , fast and slow. In the fast test the maze is held vertically upright under a source of light and run for two minutes. The flies move upwards driven by geotaxis and phototaxis. In the slow test, the maze is placed horizontally in a darkened room and run for fifteen minutes. The fast test can only be used to study responses to repellents. To measure responses to attractant stimuli starved flies are subjected to the slow chemotaxis test.

In both cases, about 100 flies are introduced into the start tube (Fig 1A) and allowed to distribute themselves in the maze. After a fixed time the number of flies in the smell and control tubes is counted. The flies are tapped back into the start tube and the test repeated after rotating the maze through 180° so that the positions of the odor and control arms are interchanged. Each batch of flies is tested four times and the Response Index (R.I.) calculated from the sums of the four trials.

4.Calculation of Response Index

The response Index is defined as the difference of the number of flies in the odor(S) and control(C) arms of the maze divided by the sum of the flies in the two arms. $R.I.=(S-C)/(S+C)$. +1 indicates total attraction and -1 indicates total repulsion. In the fast test, nearly 95% of the flies move out of the start tube. In slow chemotaxis, about 20% of the flies remain behind in the start tube. These flies are excluded from the calculation of the R.I.

All glassware for olfactometry are washed in scent free detergent and dried in an oven at 200^{o} C. Fresh polyethylene tubing is used for each concentration of the odorant.

Jump assay: A modification of the test described by Helfand and Carlson (1989) was used. The test measures the ability of flies to jump in response to exposure to an air stream bearing an odorant. The apparatus used is shown in Fig. 1B. Air is blown through the odor solution into the jump tube at the rate of 1 liter per minute.

Two to four day old flies are transferred onto fresh culture medium 24hrs. prior to the test. They are immobilized on ice and introduced singly into the glass "jump" tubes plugged with cotton wool stoppers. The flies are left in the tubes for at least half an hour before testing to allow for recovery from anesthesia. The tube is connected to the apparatus and the air flow turned on only when the fly reaches two-thirds of the way up the tube. A jumping event is scored if the fly hits the cotton stopper 5 secs after the flow is begun. A fresh tube is used for each test.

Typically a group of 20 flies is tested individually and the group score defined as the percentage of flies that jumped.

Control flies are tested in parallel with each experimental set. In each experimental set, flies are tested against water instead of odorant to test the noise level of jumps in response to air currents or humidity.

Mutagenesis.

Mutagenesis with ethanemethylsulfonate (EMS) was carried out according to the procedure of Lewis and Bacher,(1955). Isogenic X-chromosome lines were set up with C(1)DX yf virgins either directly or after enriching the F1 for mutants, either at the larval or adult stages.

Enrichment at the larval stage involved running animals in the plate assay against 10^{-2} dilution of iso-amyl acetate. At this concentration, normal larvae are strongly attracted and appear on the smell half of the plate (R.I.=0.8±0.07) Larvae found on the control half after 5 minutes of test were collected and placed in vials containing medium. The emerging males were used to set up lines.

To enrich for mutants at the adult stage, F1 progeny were fractionated in an olfactometer against a 10^{-3} dilution of benzaldehyde. This concentration is strongly repellent to adult flies eliciting an R.I. of -0.90.06 from CS flies.

Males recovered from the smell arm of the maze were collected and used to set up lines.

Meiotic Mapping

For a confident classification of recombinants emerging from a cross, pure lines have to be set up and tested. This makes large scale testing of randomly chosen progeny an arduous task. We have therefore carried out genetic mapping of the olf genes in two steps.

Mutant males were crossed to females bearing the visible markers yellow(y1.00), crossveinless (cv1-13.6), vermillion((v 1-33.0), forked (f1-56.0) and carnation(car 1-62.5). The F1 progeny were selved. Recombinant males in the F2 carrying single exchanges were selected for setting up pure lines. These lines were classified for their olfactory behavior. Mapping by recombinant selection permitted an approximate localization of the olfactory gene in the marked interval.

For a closer mapping of the mutations between cv and v, the olf mutants were crossed to the strain carmine(cm1-18.9), cut(ct 1-20.0) and singed(sn1-21.0). Recombinants between the two intervals cm and ct and ct and sn were selected and pure lines set up and scored for their olfactory responses.

Cytogenetic Mapping

The deficiencies and duplications used are listed in Table 1 and Figure 8. The relevant heterozygotes were tested for olfaction in either the Y-maze or the jump assay.

RESULTS

Olfactory Responses of the Wildtype

Drosophila has an exquisite sense of smell and the responses of the larva as well as the imago can be measured quantitatively in normal or mutant flies. At the outset we examined the behavior of the wildtype to over 50 different volatiles which are either constituents of food or known to attract other insects. The strongest responses were confined to a small subset of odorants (Rodrigues, 1980; Siddiqi, 1983). Very often members of the same chemical group elicited unequal responses. Thus ethyl acetate, iso-amyl acetate and n-butanol were strong attractants, but citronellyl acetate or geraniol were relatively ineffective. Benzaldehyde was a strong repellent other aldehydes repelled weakly. We then chose a set of six odorants representing the main classes of compounds that evoked a prounced response from Drosophila. The behavior of normal and mutant strains described in this paper is based on experiments with the six odorants- ethyl acetate, iso-amyl acetate, n-butanol, cyclohexanone, benzaldehyde and propionic acid.

The larvae do not exhibit any avoidance reactions in the plate test. At lower concentrations of the odorants they are attracted by all the above-mentioned volatiles as well as some apparently noxious chemicals such as pyridine (Fig. 2; Rodrigues, 1980). In the case of the latter category the larvae do not run into the disc but form a ring around it. The diameter of the ring is proportional to the concentration of the source (Rodrigues, 1980). At elevated concentrations of the odorant, the larva makes a bee-line towards the source, but with decreasing strength of the cue its tracks begin to approximate biased wandering (Aceves-Pina and Quinn, 1979: our unpublished observations).

A variety of olfactometers have been employed by different investigators to characterize the response of the imago. The sensitivity of the odorant is measured either in terms of the threshold concentration or the maximal response. The exact values of these indices depend upon the design of the olfactometer and the "state" or "disposition of the fly. Y-mazes which introduce odors in the air stream give lower detection thresholds than mazes without air flow. The maze employed by us is easy to assemble and provides relatively rapid measurements. As described in the section on methods, the optimal conditions for measuring attraction and repulsion are not the same. Repulsion is best measured in rapid tests when alarmed flies driven by geotaxis are forced to make a binary choice. On the other hand attraction is most evident when starved flies are allowed to explore the maze relatively at ease.

Figure 2 shows the response curves of two to four day old flies against the six test chemicals employed in these experiments. Unlike the larva, the imago can discriminate between repellents and attractants. Benzaldehyde is the strongest among the repellents; it is avoided at very low concentrations. The most potent attractants are the acetate esters ethyl acetate and iso-amyl acetate. The attractants elicit a mixed response, changing from attraction to repulsion beyond a certain concentration. Thus odorants cannot simply be described as attractants or repellents without reference to their concentration . Nor can it be assumed that the behavioral response at different concentrations employs identical mechanisms. This is important when considering possible explanations of altered responses in mutants.

Isolation of Olfactory Mutants.

In an experiment reported previously (Rodrigues and Siddiqi, 1978; Rodrigues, 1980), 1054 unenriched lines were screened against benzaldehyde and *Drosophila* food. In all, 10 mutants were isolated which led to the identification of four X-linked genes *olfA*, *olfB*, *olfC* and *olfD*. Defects in *olfA* and *olfB* affect the fly's ability to detect benzaldehyde; *olfC* mutants altered the responses to acetate esters and *olfD* rendered the flies anosmic altering responses to several classes of chemicals.

Later mutageneses were designed to enrich lines for mutants in behavioral tests. 9,700 F1 male progeny from mutagenized flies were fractionated in the olfactometer against 10^{-3} dilution of benzaldehyde. 87 lines were set up from the males recovered from the smell arm of the maze. On re-testing against benzaldehyde four mutants were confirmed. All four mutants were recessive and X-linked. Males from each of the four strains were crossed to olfA^{x1} olfB^{x4} and olfD^{x9} and the heterozygotes tested for their responses to benzaldehyde. In addition heterozygous combinations between the four new isolates were also generated and tested.

Two of these mutants x23 and x24 failed to complement olfA^{x1}. x26 and x27 complemented olfA, olfB olfC as well as each other. These were placed in two new complementation groups, olfE and olfF.

The ease and reproducibility of the larval plate assay makes it an attractive screen for the enrichment of mutagenized progeny for olfactory mutants. We used 10^{-2} dilution of iso-amyl acetate to fractionate 26,000 F1 larvae from mutagenized flies. Larvae found on the control halves of the plate were collected and grown up to adults. The males were crossed to C(1)DX yf females. 37 lines were set up and on re-testing two mutant lines were confirmed. Both these strains failed to complement the olfC^{x3}..

Characterization of Olfactory loci.

A. Aldehyde mutants.

A total of ten mutants have been isolated in two independent experiments. In all these strains, the responses of both larvae and adults to benzaldehyde was reduced. One of these olfDx9, is distinct from the others in showing abnormalities in responses to several other classes of odorants apart from aldehydes. The other ten are similar in that they show a reduction, rather than a total inability to respond to benzaldehyde. Complementation analysis between these strains places the ten mutants into four complementation classes. The phenotypes and genetics of these classes are described below.

olfA All six alleles are recessive and show only a partial reduction in their responses to benzaldehyde at both, the larval and the adult stages. The alleles designated x1, x6, x8 and x11 were isolated in a screen of unenriched lines. x24 and x25 were obtained after testing of lines set up after fractionation against benzaldehyde as described. All alleles showed normal viability and fertility when reared at 22-25 C. Figure 3 shows the responses of olfA^{x1}, one of the stronger alleles, to benzaldehyde. When tested in the olfactometer against benzaldehyde, mutant flies did not detect 10^{-5} dilution of benzaldehyde. Wildtype flies detect benzaldehyde at concentrations as low as 10^{-8}. In the jump assay(data not shown), mutant flies show a 50% decrease in response as compared to the wildtype strain when tested against undiluted benzaldehyde. (CS=88.5±11.5% olfA=47.5±10.8)

The larvae do not show a significant change in the threshold of detection but show an 80% reduction in the maximal response of the larvae compared to the wildtype. Larvae and adults also show a decreased response to salicylaldehyde and formaldehyde similar to that shown to benzaldehyde, while responses to ethyl acetate, acetone, acetic acid and ethanol are comparable to those of the wild type(data not shown)

In order to check the penetrance of the mutation, flies were run in the maze against 10-4 benzaldehyde. Mutant flies give an R.I. of -0.17±0.03(n=7). Flies from the smell and control arms were collected separately and re-tested against this stimulus. The populations collected from the smell and control arms of the maze gave indices of -0.19 and -0.15 respectively. The data suggests that the population does not consist of a low and high responding sub-population, but rather than all flies are affected to the same extent by the mutation. The mutation affects responses of male and females to a similar extent.

$\underline{olfA}^{x1}$ was localized on the X-chromosome with respect to the visible markers,, y, cv, v f and car (see methods) Males from the progeny showing recombination in each of the intervals were selected and lines set from these by crossing to C(1)DX virgins. The progeny of these lines were tested for olfactory behavior. The linkage of these markers to olfA is shown in Table 2a. The data places olfA about halfway in the interval between cv and v. In an effort to obtain a more precise position, the meiotic mapping described was repeated with a strain bearing the closely linked markers carmine (cm 1-18.9), cut (ct1-20.0) and singed (1-21.0). From 5748 F2 male, recombinants between the cm-ct and ct-sn interval were selected and crossed to FM7 virgins to set up homozygous lines. The lines were tested for olfaction at both the larval and adult stages. Results summarized in Table 2b, place *olfA* in the *ct*-*sn* interval.

For cytogenetic mapping, *olfA*^{x1} was crossed to a set of deficiencies and duplication strains (Figure 9) which span the *ct-sn* interval. Heterozygotes against deficiencies and duplications were tested in the Y- maze against benzaldehyde (Table 2c). The response of *olfA* males bearing either duplications FN107 (7A8;8A5) or *sn*^{13a1} (6C11;7C9)) is comparable to the wild type. The strains used to generate these genotypes (Table 1) have the autosome not carrying the duplication marked with dominant markers *Ki*. Thus *olfA*/Y;*bw*^{D}/+ and *olfA*/Y;*Ki*/+ flies that arise in the progeny were tested as controls to test for effects due to the background of these strains. The results(Table 2) place the gene in the region of overlap of the two duplications i.e. 7A8;7C9. The gene does not lie within the breakpoints of the deficiencies C128 (7D1;7D5-6) or ct $^{268-42}$(7A5-6;7B8-C1) thus restricting it to the region between 7C1 and 7C9.

olfB

The extant allele -*olfB*^{x4} shows a fully recessive temperature dependent phenotype. Flies as well as larvae of this strain reared at 28.5+- 1.5°C Show a reduced response to benzaldehyde (Fig 4). A reduced response to other aldehydes such as formaldehyde and salicylaldehyde is also observed (data not shown). Larvae and adults grown at non-permissive temperatures are normal in their responses to ethyl acetate, acetic acid, ethanol and acetone. The viability and fertility of *olfB* adults reared at 28° C are comparable to that of the wild type.

olfB^{x4} is recessive and complements mutations in *olfA,* *olfE* and *olfF*. *olfB* males were crossed to multiply marked females of genotype *y*, *cv*,*v*,*f* *car*. The progeny were selved and and grown at 28°C. The F2 males were run in the Y-maze using 10^{-3} dilution of benzaldehyde as the test chemical. Flies found in the smell arm of the maze were collected at the end of the test and scored for the markers. The results shown in Table 3a place *olfB* between *y* (1.00) and *cv*(13.0). In order to confirm this localization, F2 males from the above cross showing recombination in the *y*-*cv* interval were selected and lines were set with C(1)DX virgins. The male progeny from the lines were tested for olfaction against 10^{-4} dilution of benzaldehyde. The data is summarized in Table 3b. Mapping with a set of deficiencies and duplications in the region (Fig 9; Table 3c) places *olfB* to between 2B17 and 2C2. The locus is included in deficiency dor 2T and Dp(1;3) wv^{co}, but not Df(1) wv^{co}.

The phenotype of the mutation uncovered by a deficiency is not significantly different from that of the homozygous mutation.

olfE

The mutant *olfE*^{x26} was isolated from lines established after fractionation of the progeny of EMS treated males. The extant allele is recessive and complements _olfAx1_, _olfBx4_ and _olfFx27_ (data not shown).

The larvae and adults of _olfE_^{x26} show a reduction in their response to benzaldehyde. (Fig. 5). The adults were also tested for their response to benzaldehyde in the odor-induced jump paradigm (McKenna et al. 1989). 88.5±11.5% of CS flies jump in response to undiluted benzaldehyde. Under these conditions, only 33.0±10.7% of _olfE_ adults respond. Both wildtype and mutant flies exhibited a 10% jump response to air blown through water alone. _olfE_ larvae and flies show normal sensitivity to ethyl acetate, iso-amyl acetate or propionic acid.

olfE was localized on the X chromosome as described in methods by crossing with the strain _y_ _cv_ _v_ _f_. In a preliminary experiment where only 5 lines were set up from recombinants in each interval, _olfE_ was localized to the interval between _cv_ and _v_ (data not shown). In an independent experiment, 56 lines were set up with recombinant males showing recombinations between _cv_ and _v_. The male progeny from the lines were tested in the Y-maze using 10^{-4} dilution of benzaldehyde as the stimulus. The data summarized in Table 4a places _olfE_ half way between the markers _cv_ and _v_ at approximately 23 map units.

olfE was mapped cytogenetically with the aid of a set of deficiencies and duplications spanning the 7-8 region of the X chromosome (Fig. 9). The _olfE_ defect is by covered

X chromosome (Fig. 9). The olfE defect is by covered duplication of the region 7A8 to 8A5 in Dp(1;2)FN107 (Table 4b). Heterozygotes of olfE with Df(1) snC128 exhibit a mutant phenotype placing the gene in cytogenetic position 7D1-7D5-6.

olfF.

Only one allele of this gene exists. It was isolated after fractionation of the F1 of EMS treated flies in the Y-maze against benzaldehyde. The mutant is recessive and complements $olfA^{x1}$, $olfB^{x4}$ and $olfE^{x26}$. Homozygous females of the original isolate were sterile; the eggs laid by these females were flaccid with poorly formed chorions.. Subsequent analysis showed that the egg shell defect did not segregate with the olfactory defect; it was due to mutation of a distinct X-linked gene in the same strain.. Adults of olfF show an elevated threshold of detection of benzaldehyde. These flies show no response to benzaldehyde at 10^{-5} dilution measured in the Y-maze. At this concentration, CS respond with an R.I. of -0.15±0.05. At 10^{-4} dilution of benzaldehyde, olfF responds with an R.I. of -0.14±0.04 (CS R.I.=-0.34±0.03). There is no significant difference between males and females (Table 5). Responses of olfF to ethyl acetate, iso-amyl acetate, propionic acid and butanol are comparable to those of the CS strain.

olfF males were crossed to females bearing the X-linked visible markers and lines representing the recombinant summarized in Table 5a places olfF in the interval between y and cv. Mutant males were crossed to females bearing deficiencies and duplications in the y-cv interval (Fig.9). The olfactory gene is included in Df(1)wv^{co} and Df(1)64C18 and is covered by Dp(1:3) wv^{co} (Table 5b). The eggs laid by olfF/Df(1)wv^{co} were normal in morphology and fertile.

B Ester mutants.

The acetate esters, especially ethyl acetate and iso-amyl acetate are strongly attractive to *Drosophila*. Mutants exhibiting reduced responses to esters were isolated in two independent mutagenesis experiments. Four alleles- x2, x3, x5 and x10 were isolated by testing a set of 1054 X chromosome lines in an olfactometer using fermenting *Drosophila* medium as a stimulus. Subsequent testing showed that all these alleles also exhibited a reduced response to an ester iso-amyl acetate. In a second experiment, we fractionated larvae against iso-amyl acetate and isolated two X-linked mutants, both of which failed to complement olfC^{x3}

The six olfC alleles are all fully recessive and can be grouped into two classes depending on their behavioral phenotypes. When the responses were measured in the slow chemotaxis assays (see methods) x2, x3 and x10 showed a reduced attraction to both ethyl acetate and iso-amyl acetate compared to the wild type. x5, x14 and x17 appear to detect ethyl acetate normally but showed a reduced response to iso-amyl acetate. The responses of the strongest allele of each phenotypic class are given in Fig 6. The effect on the larvae is also strongest in these alleles, although the other alleles also show a reduced larval response. *olfC*^{X3} and *olfC*^{X17} were also tested for their response to both acetates in the odor-induced jump paradigm. *x3* is impaired in response to both iso-amyl acetate and ethyl acetate while *x17* shows a reduced response to iso-amyl acetate with no effect on the ethyl acetate response (Table 6a) Larvae and adults of *olfC*^{x3} and *olfC*^{x17} exhibit normal responses to benzaldehyde, butanol and propionic acid (data not shown.) Heterozygotes between the two phenotypic groups of alleles are defective in their responses to both iso-amyl and ethyl acetate (Table 6b)

olfC^{x10} was localized by recombination mapping to the interval between *cv* and *v* (Table 7). In a second experiment, the F1 females of a cross between *olfC x10* and *cm ct sn* were backcrossed to the recessive parent. 4228 of the progeny were screened and lines set up with males showing recombination in the *cm*-*ct* and *ct*-*sn* intervals. The pure lines were tested for their olfactory responses in the Y-maze against iso-amyl acetate and also at the larval stage in the plate assay. The results shown in Table 7 place *olfC* to the right of *ct* .

olfC^{x3} and *olfC*^{x17} were tested for complementation with a set of deficiencies and duplications in the *sn* region (Fig 9). The mutation was found to be uncovered by Df(1) sn C128 and covered by Dp(1;2)FN107, placing the gene in the interval 7D1-

7D5-6. (Table 6: Fig.9) The phenotype of olfC^{x3}/deficiency is not significantly different from that of the homozygous mutant. On the other hand, olfC^{x17}/deficiency is defective in response to both ethyl and iso-amyl acetates unlike the homozygous olfC^{x17} which responds normally to ethyl acetate.

Rodrigues (1980) and Siddiqi (1983) had investigated mutual interferences between different pairs of odorants in behavioral and electrophysiological experiments. These experiments suggested that there are more than one independent channels for the reception of ethyl acetate and iso-amyl acetate. The existence of two distinct phenotypic groups at the olfC locus is consistent with our earlier finding. It raises the possibility that the response to acetates could be further reduced by additional mutation. olfC^{x17} which is partially blocked to iso-amyl acetate was subjected to a second round of mutagenesis.

olfC^{x17} males mutagenized with EMS were mated to C(1)DXyf females. 14,000 F1 larvae were fractionated against a 10^{-2} dilution of iso-amyl acetate on a Petri plate. After two rounds of fractionation 65 non-responding males were selected to set up pure lines. Subsequent testing showed that these strains had an olfactory deficit stronger than that of x17. One of these strains designated olfC^{x17-1a} has been analyzed further. Figure 7 and Table 8 show the olfactory responses of olfC^{x17-1a}. The defect in the response to iso-amyl acetate

How many?

is considerably stronger than in olfCx17. In addition x17-1a shows a reduction in response to benzaldehyde. There also appears to be some reduction in the ethyl acetate response. The "double mutant" x17-1a is fully recessive.

The defect in sensing of both benzaldehyde and iso-amyl acetate is covered by the duplication FN107 and uncovered by deficiency ct^{J6} placing 1a in 7A2-3;7C1 (Fig. 9).

C Multiply blocked mutant $olfD^{x9}$.

The anosmic mutant $olfD^{x9}$ was obtained from EMS mutagenized lines (Rodrigues and Siddiqi, 1978). The original isolate showed very poor viability which improved somewhat by repeated outcrossing. Two lethal alleles of olfD (L1 and L2) were isolated after crossing mutagenized males to olfD females. Lilly and Carlson (1990) subsequently found that olfD is an allele of smellbind (sbl) a gene first described by Aceves-Pina and Quinn (1979).

olfD is a strong smell-blind mutant which is simultaneously blocked to benzaldehyde, ethyl acetate iso-amyl acetate, acetic acid, propionic acid, ethanol and butanol. The larval responses to some of these odorants is shown in Figure 8.

Preliminary meiotic crosses placed $olfD^{x9}$ in the interval between cv and v (unpublished). $olfD^{x9}$ was crossed to the triply strain cm ct sn. Scoring of the olfactory behavior of selected recombinants in the cm-ct and ct-sn intervals indicated that *olfD* mapped to the right of *sn* (Siddiqi, 1987).

Lilly and Carlson (1990) have recently carried out an extensive genetic analysis of the locus *sbl*. They have located it to the right of *vermilion* at 14F6;15A4. Our earlier placement of *olfD* to the left of *v* is therefore erroneous. In view of the results of Lilly and Carlson (1990), *olfD* has been re-designated as *sbl*^{Dx9}.

DISCUSSION

The olfactory pathway of Drosophila provides a well mapped and tractable system for a neurogenetic analysis of olfaction (Siddiqi, 1987). The organization of the sensillae and their connections to the brain are relatively simple. The sensory hairs contain two to four neurons which project to the 22 glomeruli of the antennal lobe (Stocker et al, 1983; Venkatesh and Singh, 1984; Pinto et al, 1988). The sensory neurons can be classified into a small number of recognizable types which carry overlapping sets of acceptor sites (Siddiqi, 1987). Functional mapping with ^{3}H-2-deoxyglucose indicates that individual odors project to discrete sub-sets of glomeruli (Rodrigues and Buchner, 1984; Rodrigues, 1988).

Barrows (1907) showed that Drosophila detects odors through its antenna. This was followed by the work of W.H. Thorpe (1939) on olfactory conditioning and of Begg and Hogben (1946) on the antennal mutants. The advent of Drosophila neurogenetics in the sixties revived interest in olfactory behavior. Becker (1970) selected repellent insensitive strains and Kikuchi (1973) described a mutant strain that was attracted by several odors that repel the wild type. We described the first X-linked olfactory mutations blocking the responses to aldehydes or acetates (Rodrigues and Siddiqi, 1978; Rodrigues, 1980). More recently additional olfactory loci have been described by John Carlson and his associates (Helfand and Carlson,1989; McKenna et al, 1989; Woodward et al, 1989).

The four aldehyde genes olfA, olfB, olfE and olfF reduce repulsion to a group of aldehydes; these mutants respond normally to acetates, alcohols, fatty acids and ketones. The mutants of the olfC group show reduced attraction and, except for a slight~~ly~~ deficiency in acetone response in olfC^{x10} (Rodrigues, 1980), exhibit normal behavior towards other chemicals. Mutations in the six olf genes affect the imago as well as the larva. Figure 9 summarizes the cytogenetic localisation of the olf genes and the breakpoints used to map these loci.

The most notable feature of the aldehyde and acetate ester mutants is the specificity of their anosmia. Indeed, the olfC mutants show an even higher order of specificity; the sub-groups of alleles distinguish between ethyl acetate and iso-amyl acetate. The specificity of the block suggests that the lesion is perhaps close to the peripheral end of the reception pathway. Venard and Pichon (1984) found that the electroantennogram (EAG) of one allele of olfC is less sensitive to butyl acetate and iso-amyl acetate. The response to ethyl acetate is very slightly affected.

Aldehyde?

Currently, we are investigating the single-unit responses of sensilla basiconica in the mutant strains. A partial reduction in firing against benzaldehyde has been observed in olfA (A. Gupta and O. Siddiqi, unpublished work). The existence of a multiplicity of sensillary types, not all of which might be affected by the mutation, makes it more difficult to localize the neurophysiological correlates of partial behavioral defects.

Morever, reduced firing in the sensory neuron does not necessarily imply a defect in reception *per se*. Such an affect can also arise from a change in the mechanism of translocation or elimination of odorant molecules from the acceptor site. For the present, the nature of the primary defect in olfactory mutants remains to be identified.

Three of the *olf* genes are located in the chromosomal region between *cut* and *singed* and at least two i.e. *olfE* and *olfC* are very close to the *singed* locus. The question arises whether the region of the X chromosome around *singed* contains a family of genes concerned specifically with olfactory functions. Gaiti Hasan (1989) in our laboratory, has carried out a chromosomal walk covering this region and mapped *olfE* to a 30kB region of this walk.

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LEGENDS TO FIGURES..

Figure 1

Diagram of paradigms used to measure olfaction in *Drosophila* adults. A) Y-maze: The maze consists of a Y choice tube the arms of which are connected by ground glass joints to the odor and control arms of the maze. Air is drawn into the maze through gas bottles containing 100ml of odor solution(S) or water(C). The suction of the pump is adjusted through a set of valves and monitored through a flow meter. Flies are introduced into the maze from the start tube which is connected to the choice tube by a ground glass joint. The tubings used to connect the odor and control reservoirs are made of chemically inert teflon of I.D. 1mm.

B) The jump tube is made of glass I.D.=2cm tapered at one end to I.D.=1mm. After introduction of a single fly into each tube, it is connected to the odor containing reservoir(S) by a teflon tubing. Air is blown into the tube through a reservoir containing the odor solution (S)by an aquarium pump. The flow rate of the pump is controlled by a set of valves and monitored by a flow meter.

Figure 2

Dose-response curves of the wildtype to odors. Adult responses (upper panel) were measured in the Y-maze. The response to benzaldehyde was measured by running the olfactometer vertically for 2 mins. Attraction to all other odorants was measured after placing the maze horizontally in a darkened room for 15 mins. Dashed lines indicate the shift from attraction to avoidance at high concentrations of odorant. Larval olfactory behavior (lower panel) was measured in the plate test. Each point is based on data from at least ten different experiments. The error bars indicate the standard deviation.

Figure 3.

Response of olf^{x1} to benzaldehyde. A) Adult responses were measured in the Y-maze. Each column on the histogram represents the mean and standard deviation of ten different experiments. B) Early third instar larvae were tested in larval plate assays. Each point represents the mean and standard deviation of 8 different experiments.

Figure 4.

Response of olfB^{x4} to benzaldehyde. Cultures were reared at 22^{0}C and 28^{0}C and olfactory responses of the A)adults and B)larvae were measured against benzaldehyde. Adult values are based on 8 different experiments and those of the larvae on 6 sets of readings. The bars represent the standard deviations.

Figure 5.

Olfactory response of olfE^{x26} adults (A) and larvae (B) to benzaldehyde. The adult histograms are plotted from data from 10 different experiments. In the larval response curves, each point represents 8 independent experiments.

Figure 6.

Responses of *olfC* alleles to acetate esters. A)Adults B) Larvae. Adults as well as larvae of *x17* exhibit normal responses to ethyl acetate but reduced responses to iso-amyl acetate. On the other hand, *x3* is affected in response to both stimuli. N for adult experiments= 6; larval=8. The bars represent the standard deviations.

Figure 7.

Olfactory responses of *olfC*^{x17} and its derivative strain *olfC*^{x17}-1a to acetate esters. A) Adult responses were measured by the jump assay against $5X10^{-4}$ dilution of ethyl acetate and undiluted iso-amyl acetate. B)Larval response to iso-amyl acetate. Each point is based on 16 readings. The error bars represent the standard deviations.

Figure 8.

Larval response of olfD to different odorant stimuli. For each stimulus, data from ten different plate assays experiments was used to compute mean and standard deviation of response.

Figure 9

The *olf* genes on the X chromosome of *Drosophila*. The breakpoints of deficiencies and duplications used to map the cytogenetic locations of the genes are shown in the enlarged portion of map.

Table 1: Breakpoints and sources of deficiency and duplication strains. ref: Lindsley and Zimm 1987.

Strain	Breakpoints	Source
Df(1)dor2T/FM6	2B6;2E1-2	I. Kiss, BRC, Szeged
Df((1)w^{VCO}vf/C(1)RMywf/Y;	2C2;3C5	L. Craymer, CALTECH CA.
Dp(1;3)w^{VCO}/Ubx	2B17-C1;3C4-5	
Df(1)64c18/FM7	2E1-2;3C2	L. Craymer
Df(1)RF19/FM7	7A4-5;7B2-3	G. Lefevre, Calif. State Univ.
Df(1)ct^{J4},f;C(1)DXyf/Y;	7A2-3;7C1	Umea Stock Center. Umea, Sweden
Dp(1;3)sn^{13a1}/Ki	6C;7C-D1	
Df(1)ct268-42,y/FM4	7A5-6;7B8-C1	Umea, Sweden.
Df(1)snC128/FM6	7D1;7D5-6	G. Lefevre
Df(1)RA2,f/FM7	7D10;8A4-5	L. Craymer
T(1;2)sn^{+72d},fcar/C(1)RM yf/Y;	7A8;8A5	Bowling Green Stock Stock Center. USA
Dp(1;2)FN107/bwD	7B1;8A5	

Table 2: Mapping of the locus olfA.

a) Segregation of olfA among recombinants from a back-cross with a multiply marked X chromosome.

Marker	Olfactory Phenotype	
	Parental	Recombinant
y	38	11
cv	43	4
v	43	6
f	33	18
car	31	22

b) Linkage of olfA to the markers cm ct and sn .

Genotype	olf	olf^+
cm + +	9	0
+ ct sn	0	10
cm ct +	0	6
+ + sn	10	0

c) Deficiency mapping of olfA.

Genotype	R.I. to Benzaldehyde(10^{-4})		
	Mean	S.D.	N
Canton-Special	-0.33	0.03	14
olfA/Y	-0.16	0.03	10
olfA/olfA	-0.18	0.04	6
olfA/+	-0.38	0.08	6
olfA/Y; Dp(1;2)FN107	-0.34	0.03	5
olfA/Y; Dp(1;2)sn13a	-0.34	0.01	6
olfA/Df(1)snC128	-0.32	0.03	5
olfA/Df(1)ct268-42	-0.34	0.01	5

Table 3: Localization and deficiency mapping of olfB

a) Segregation of visible markers among olfB flies recovered in the F2 progeny of a backcross with y cv v f car.

Marker	Number showing olf phenotype.
y	18
cv	23
v	50
f	90
car	95

b) Segregation of olfB among recombinants in the interval between y and cv.

Genotype	olf	olf^+
y + + +	5	15
+ cv v f	13	7

c) Mapping of the olfB locus with deficiencies and duplications. Responses of 28°C reared flies were tested at 10^{-4} benzaldehyde.

Genotype	Response Index		
	Mean	S.D	N
Canton-Special	-0.32	0.03	14
olfB	-0.15	0.01	8
olfB/+	-0.33	0.01	5
olfB/Df(1)dor2T	-0.17	0.04	17
olfB/Df(1)wvco	-0.35	0.08	4
olfB/Y;Dp(1;3)wvco	-0.33	0.02	9
CS/Y;Dp(1;3)wvco	-0.36	0.02	6

Table 4: Mapping of olfE.

a) Segregation of olfE among recombinants showing crossover in the cv-v interval.

Genotype	olf	olf^+
y cv + +	13	11
+ + v f	13	15

b) Mapping of olfE with deficiencies and duplications. The heterozygotes were tested at 10^{-4} dilution of benzaldehyde in the Y-maze.

Genotype	Response Index		
	Mean	S.D.	N
Canton-Special	-0.34	0.03	14
olfEx26	-0.14	0.04	19
olfE/Y	-0.20	0.04	10
OlfE/+	-0.37	0.05	6
olfE/Y;Dp(1;2)FN107	-0.35	0.06	6
olfE/Df(1)snC128	-0.12	0.04	6
+/Df(1)snC128	-0.32	0.01	7

Table 5: Mapping of olfF.

a) Segregation of olfF among recombinants in a backcross with multiply marked X chromosome.

Marker	Olfactory phenotype	
	Parental	Recombinant
y	39	1
cv	31	9
v	21	19
f	11	29

b) Deficiency and duplication mapping of olfF. All the olfactory tests were done against 10^{-4} benzaldehyde in the Y-maze.

Strain	R.I.	S.D.	N
Canton-Special	-0.34	0.03	14
olfF/olfF	-0.14	0.04	19
olfF/Y	-0.15	0.05	9
olfF/+	-0.37	0.06	9
olfF/Y;Dp(1;3)wvco	-0.32	0.03	10
olfF/Df(1)64c18	-0.14	0.03	11
CS/Df(1)64c18	-0.33	0.07	10

Table 6: Responses of olfC alleles to iso-amyl acetate and ethyl acetate.

a) Response was measured in the jump assay. Each value represents ten readings.

	Percent Response	
	Iso-amyl acetate	Ethyl acetate
CS	84±7.5	68±11.7
olfC^{x3}	47.5±7.8	27.5±7.0
olfC^{x17}	53.0±6.5	63.5±4.8

b) The responses of flies to 10^{-4} dilution of iso-amyl acetate and 10^{-3} dilution of ethyl acetate in the Y-maze. Each value represents data from at least 6 experiments.

	Response Index	
	Iso-amyl acetate	Ethyl acetate
CS	0.71±0.05	0.69±0.06
x3/x3	0.33±0.06	0.35±0.04
x5/x5	0.30±0.07	0.680.06
x14/x14	0.28±0.06	0.75±0.07
x17/x17	0.35±0.07	0.76±0.06
x3/x5	0.29±0.05	0.32±0.07
x3/x17	N. D.	0.32±0.06
x5/x10	0.33±0.06	0.35±0.07
x5/x14	0.39±0.05	0.70±0.09
x5/x17	0.28±0.04	0.68±0.07
x10/x14	0.34±0.03	0.37±0.08
x10/x17	0.29±0.04	0.37±0.05
x14/x17	0.29±0.06	0.73±0.04

Table 7: Mapping of olfC. Homozygous lines were tested at both the larval and adult stages.

a) Segregation of olfC^{x10} with visible markers on the X chromosome.

Marker	Olfactory phenotype	
	Parental	Recombinant
y	18	7
cv	18	0
v	15	3
f	13	7
car	13	12

b) Linkage of olfC^{x3} to the markers cm ct and sn.

Genotype	olf	olf^{+}
cm + +	6	0
+ ct sn	0	15
cm ct +	17	0
+ + sn	0	8

c) Deficiency mapping of olfC. 10^{-4} amyl acetate and 10^{-3} ethyl acetate was used for testing in Y-maze. N>6 measurements.

Genotype	Response Index	
	Iso-amyl ac.	Ethyl acetate
Canton-Special	0.70±0.06	0.73±.06
olfCx3/olfCx3	0.33±0.06	0.35±0.04
olfCx3/+	0.62±0.06	0.69±0.01
olfCx17/+	0.61±0.03	0.57±0.02
olfCx3/Df(1)snC128	0.30±0.03	0.37±0.04
olfCx17/Df(1)snC128	0.31±0.02	0.36±0.02
+ /Df(1)snC128	0.71±0.03	0.70±0.03
olfCx3/Y;Dp(1;2)FN107	N.D.	0.87±0.06
olfCx17/Y;Dp(1;2)FN107	0.73±0.04	N.D.
+ /Y;Dp(1;2)FN107	0.61±0.03	0.82±0.04

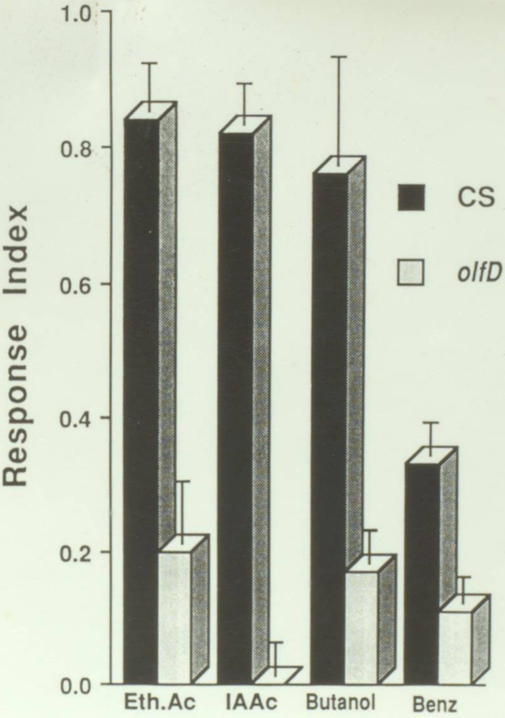
Table 8: Genotypic characterization of olf^{x17-1a}.

a) The genotypes were tested in the jump test against undiluted benzaldehyde and iso-amyl acetate. Each value is based on data from ten set of twenty flies each. In the cases marked with * responses were measured in a Y-maze against 10^{-4} benzaldehyde (n=6).

Genotype	PERCENT RESPONSE	
	benzaldehyde	iso-amyl acetate
CS	88.5±11.5	84±7.5
olfC^{x17}	90.0±10.1	53±6.5
olfC^{x17-1a}	32.0±14.2	33.5±5.8
olfC^{x17-1a}/+	82.5±10.8	83.5±11.5
olfC^{x17}/Df(1)C128	N.D.	36.6±8.8
olfC^{x17-1a}/Df(1)C128	*0.39±0.05	37.5±11.3
CS/Df(1)C128	*0.33±0.05	80.08.1
olfC^{x17-1a}/Y;Dp FN107	*0.35±0.05	86.0±5.4
olfC^{x17-1a}/Df(1)ct^{J4}	33.5±11.5	21±9.1
olfC^{x17}/Df(1)ct^{J4}	80.8±7.5	82.5±9.7

b) Response of larvae and adults to ethyl acetate. Larval responses were measured in the plate assay to 10^{-4} dilution. Flies were tested in the jump assay against $5X10^{-2}$ dilution.

Genotype	R.I. Larvae	Percent Response
CS	0.74±0.07	68±11.7
olfC^{x17}	0.60±0.03	63.5±4.8
olfC^{x17-1a}	0.48±0.1	51.5±4.9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0.4
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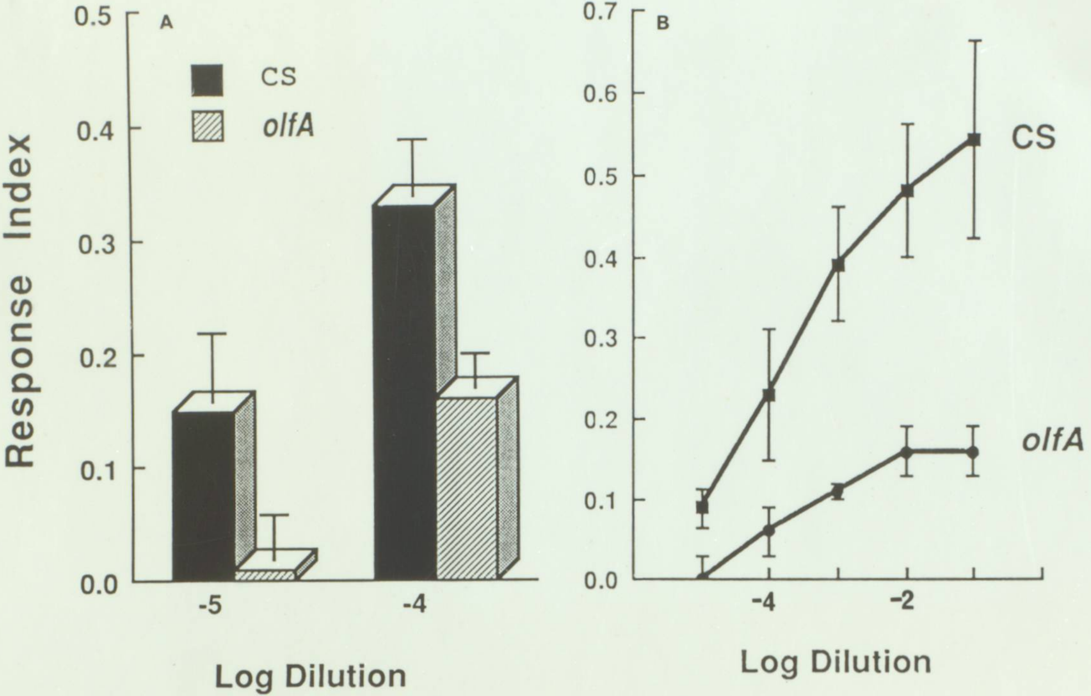
olfD
Eth.Ac
IAAc
Butanol
Benz



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CS
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0.3
0.2
0.1
0.0
-5
-4
Log Dilution
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0.4
0.3
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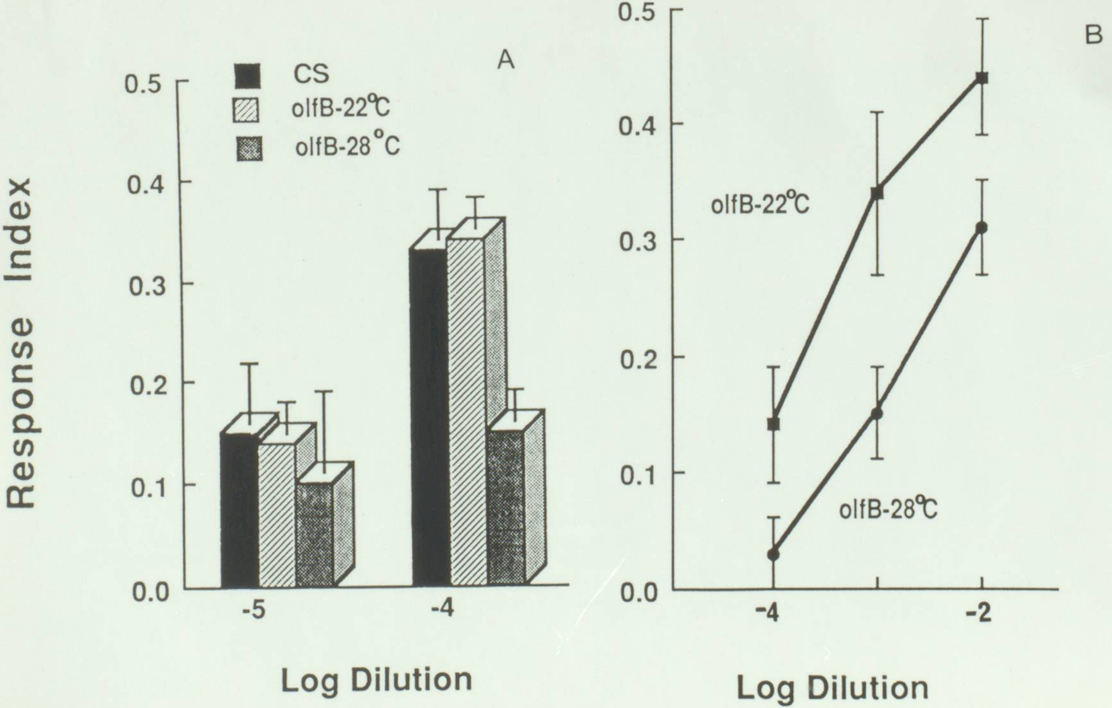
0.1
0.0
CS
olfA
-4
-2
Log Dilution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olfB-28°C
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-4
Log Dilution
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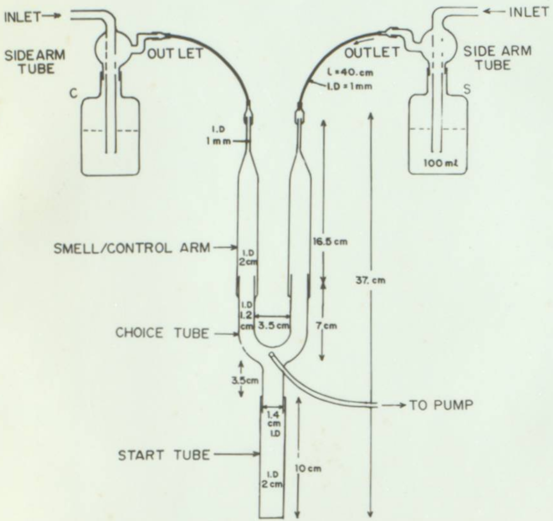
0.4
0.3
0.2
0.1
0.0
B
olfB-22°C
olfB-28°C
-4
-2
Log Dilution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INLET
SIDEARM TUBE
OUTLET
OUTLET
SIDE ARM TUBE
C
S
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I.D=1mm
I.D 1mm
100 ml
SMELL/CONTROL ARM
I.D 2cm
16.5 cm
37. cm
I.D 1.2 cm
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TO PUMP
1.4 cm I.D
START TUBE
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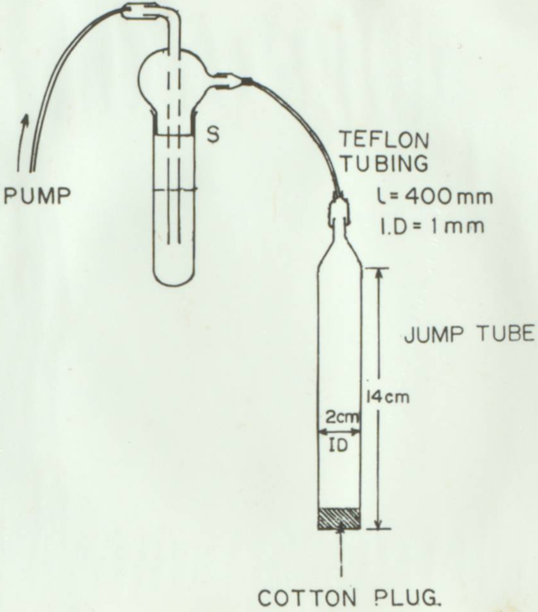
10 cm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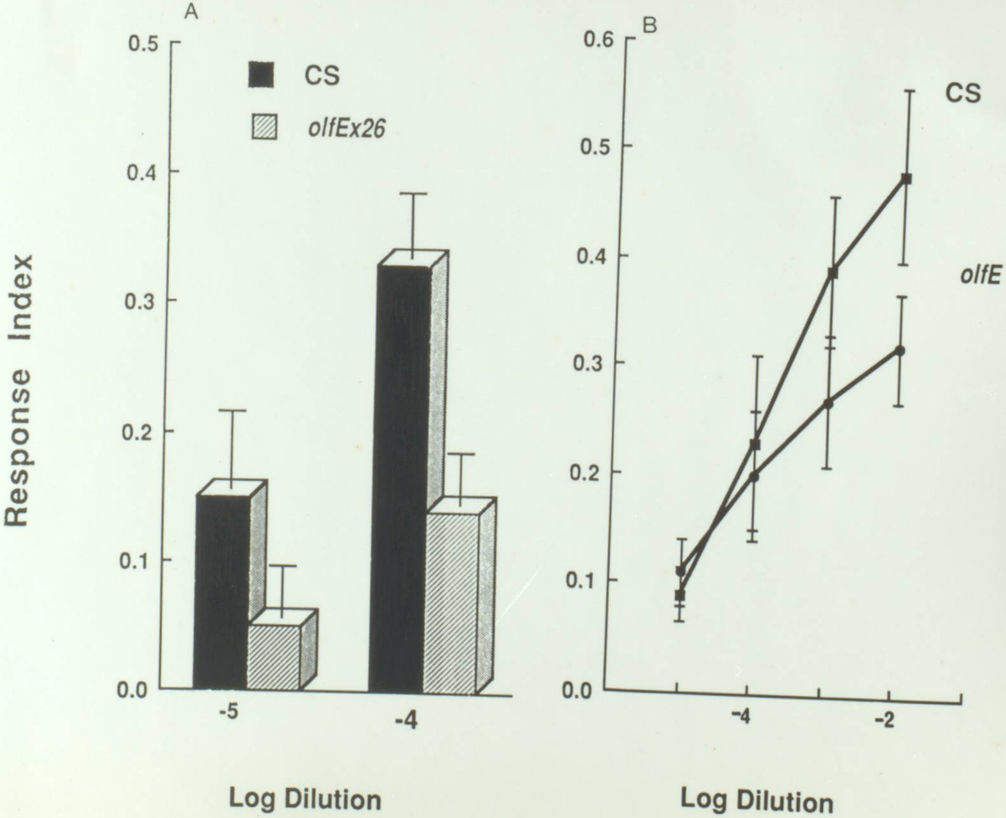
ID
COTTON PLUG.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B
Response Index
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0.4
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0.6
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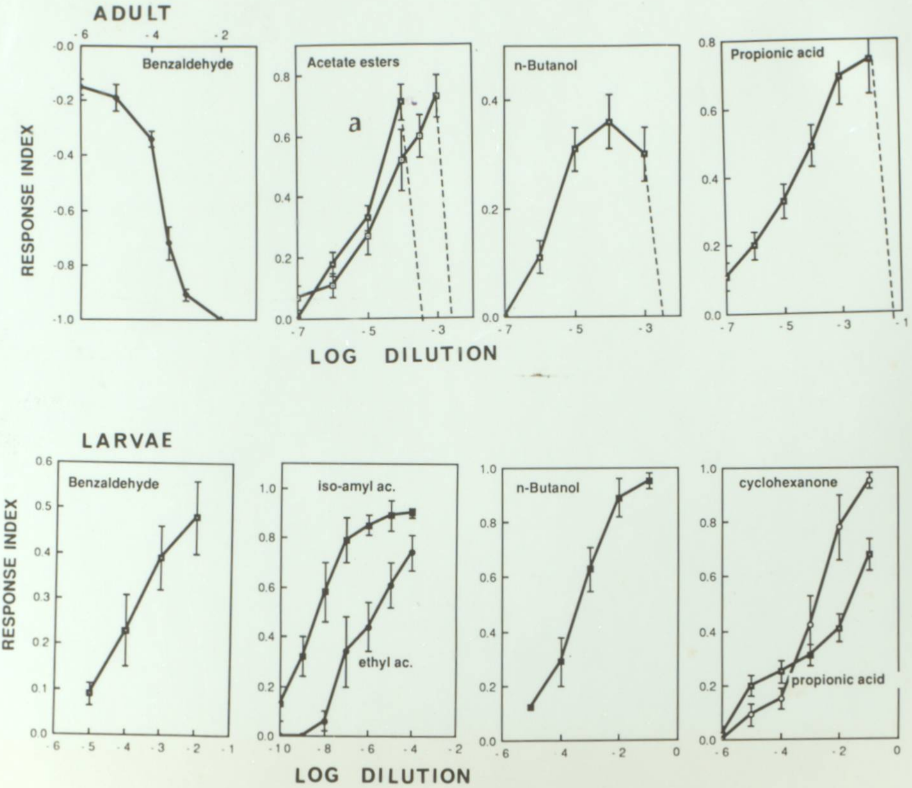
-2
Log Dilution
Log Dilution


ADULT
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-0.6
-0.8
-1.0
-6
-4
-2
Acetate esters
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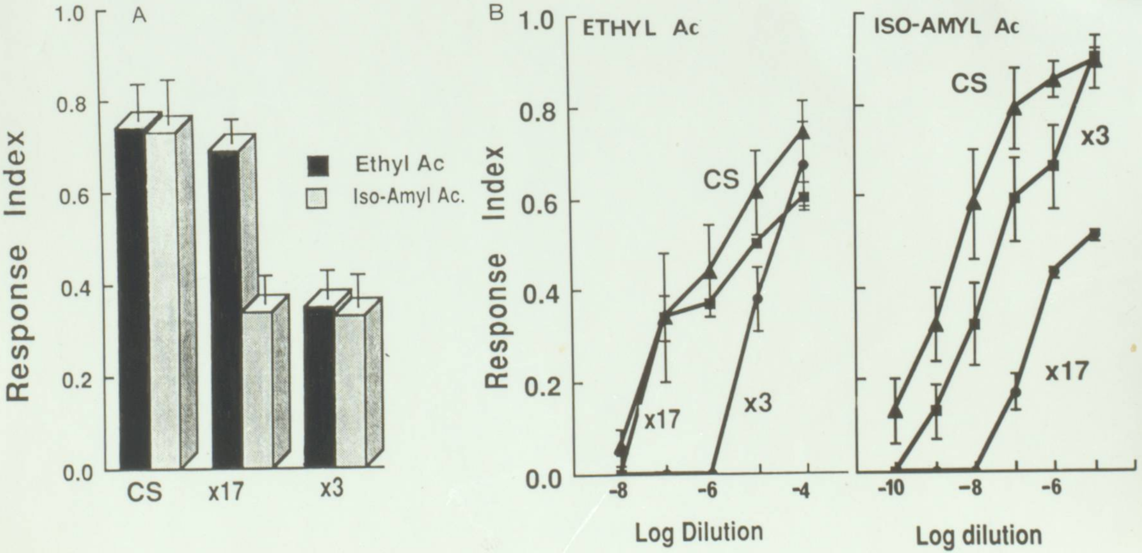
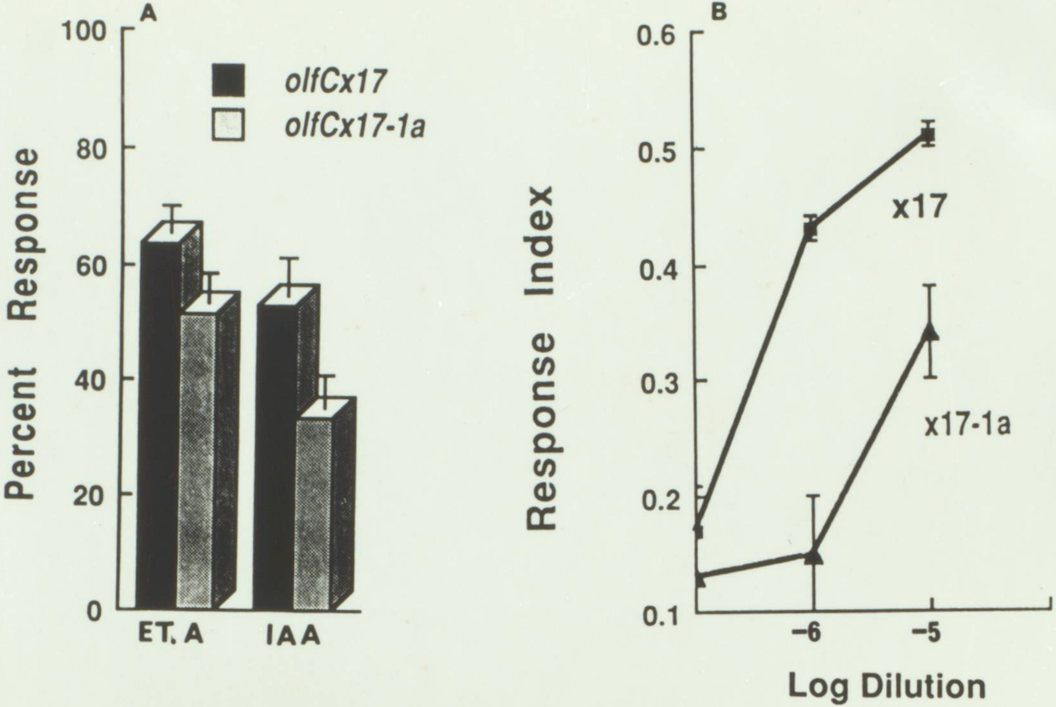
0.2
0.4
0.6
0.8
n-Butanol
0.0
0.2
0.4
Propionic acid
0.0
0.2
0.4
0.6
0.8
-7
-5
-3
-1
LOG DILUTION
LARVAE
RESPONSE INDEX
Benzaldehyde
0.0
0.1
0.2
0.3
0.4
0.5
0.6
-6
-5
-4
-3
-2
-1
iso-amyl ac.
ethyl ac.
0.0
0.2
0.4
0.6
0.8
1.0
-10
-8
-6
-4
-2
n-Butanol
0.0
0.2
0.4
0.6
0.8
1.0
-6
-4
-2
0
cyclohexanone
propionic acid
0.0
0.2
0.4
0.6
0.8
1.0
-6
-4
-2
0
LOG DILUTION

Fig 2

↑ Ayyub *et al.*

A
B
Response Index
1.0
0.8
0.6
0.4
0.2
0.0
CS
x17
x3
Ethyl Ac
Iso-Amyl Ac.
ETHYL Ac
ISO-AMYL Ac
CS
x17
x3
CS
x3
x17
-8
-6
-4
-10
-8
-6
Log Dilution
Log dilution



A
100
80
60
40
20
0
Percent Response
olfCx17
olfCx17-1a
ET. A
IAA
B
0.6
0.5
0.4
0.3
0.2
0.1
Response Index
x17
x17-1a
–6
–5
Log Dilution

One or Two concentrations?

Try both.

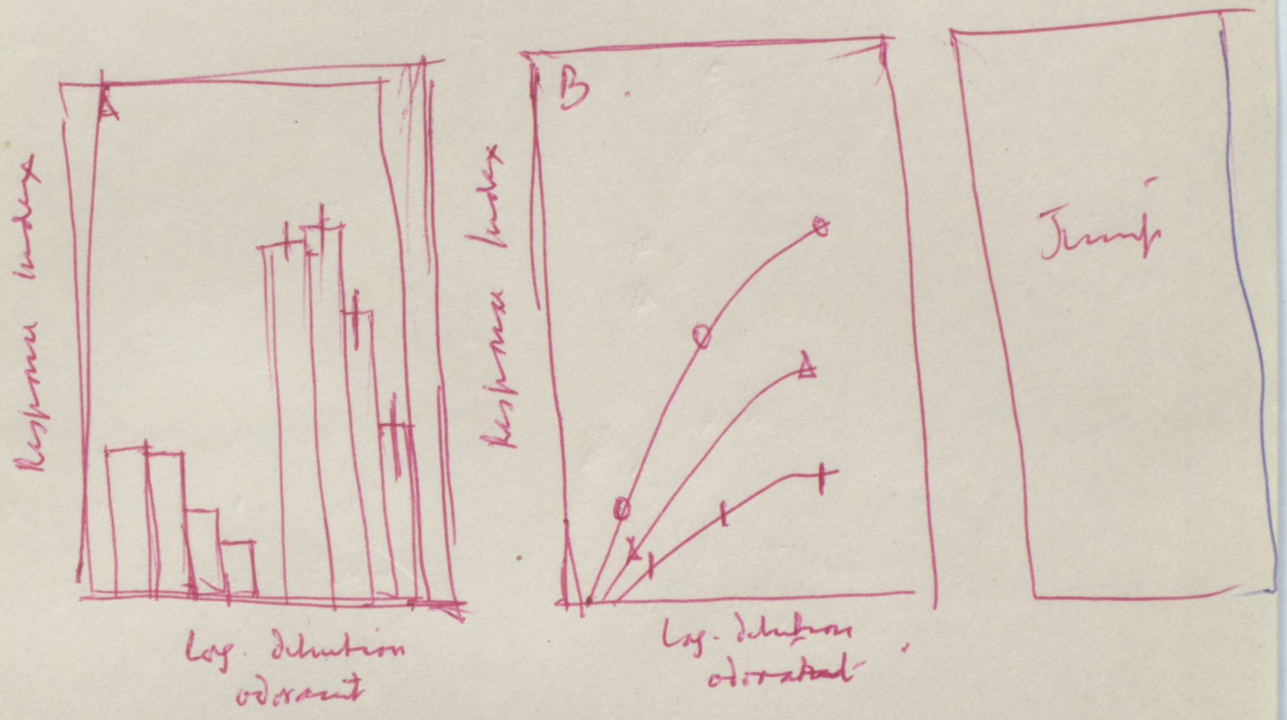
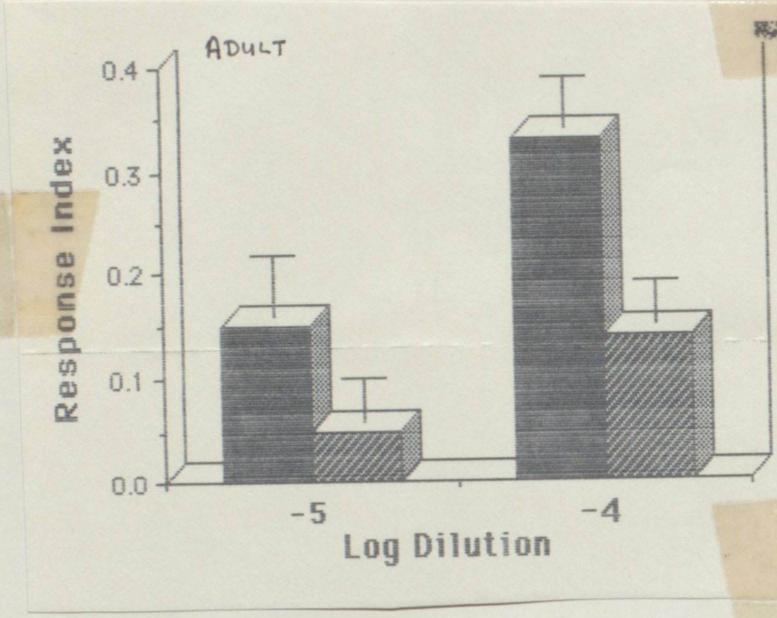
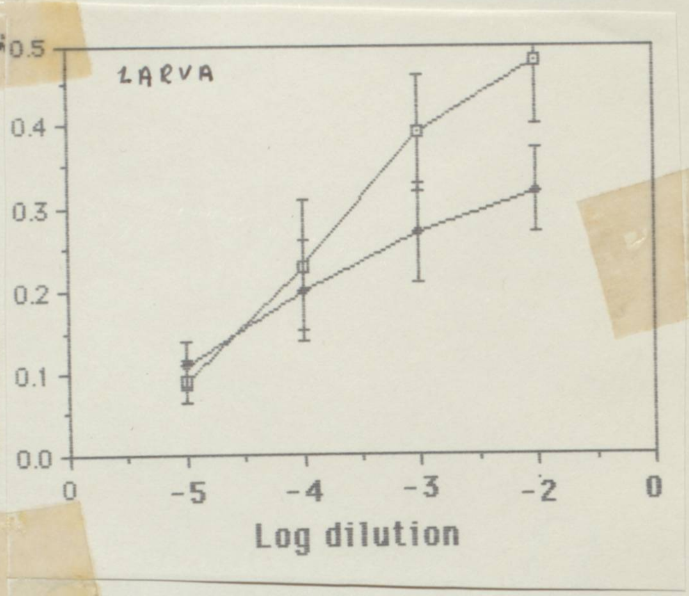


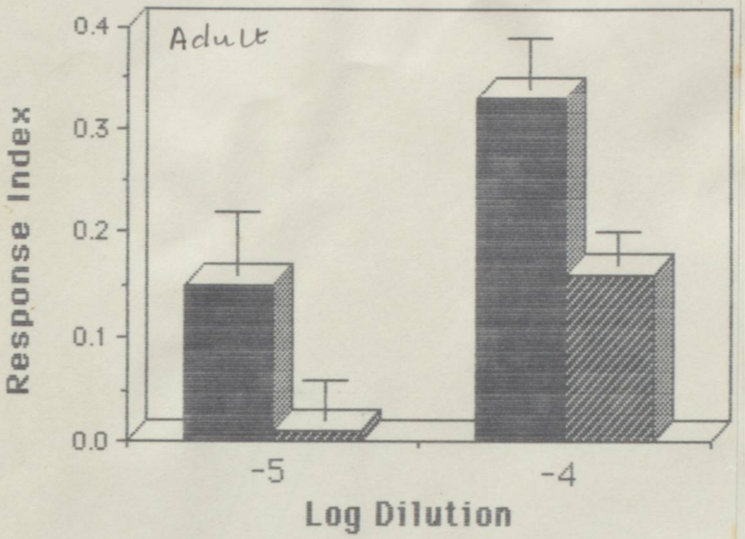
Fig 4. Response of olf Ex? to benzaldehyde.
A. Imago in Y maze :- ~~B. Larval response~~ Wild type (   ) ; olf E (   ) ; B. Larval response, wild type (   ) ; olf E (   ),

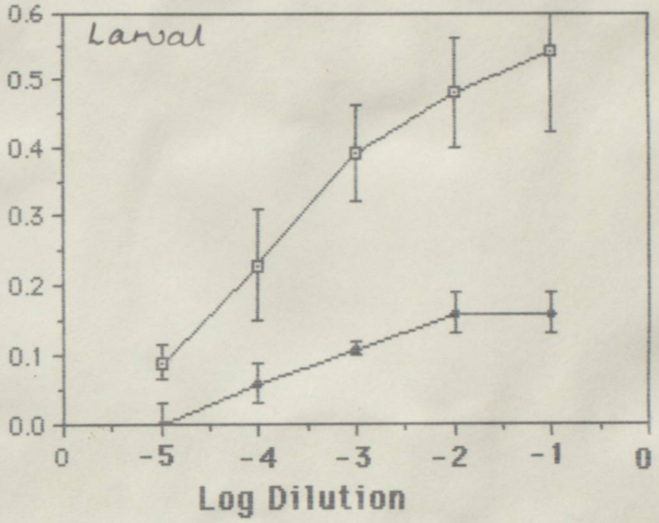




(jump test data
to be completed)

Olf E.


Adult
Response Index
0.0
0.1
0.2
0.3
0.4
-5
-4
Log Dilution


Larval
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
-5
-4
-3
-2
-1
0
Log Dilution

Olf A

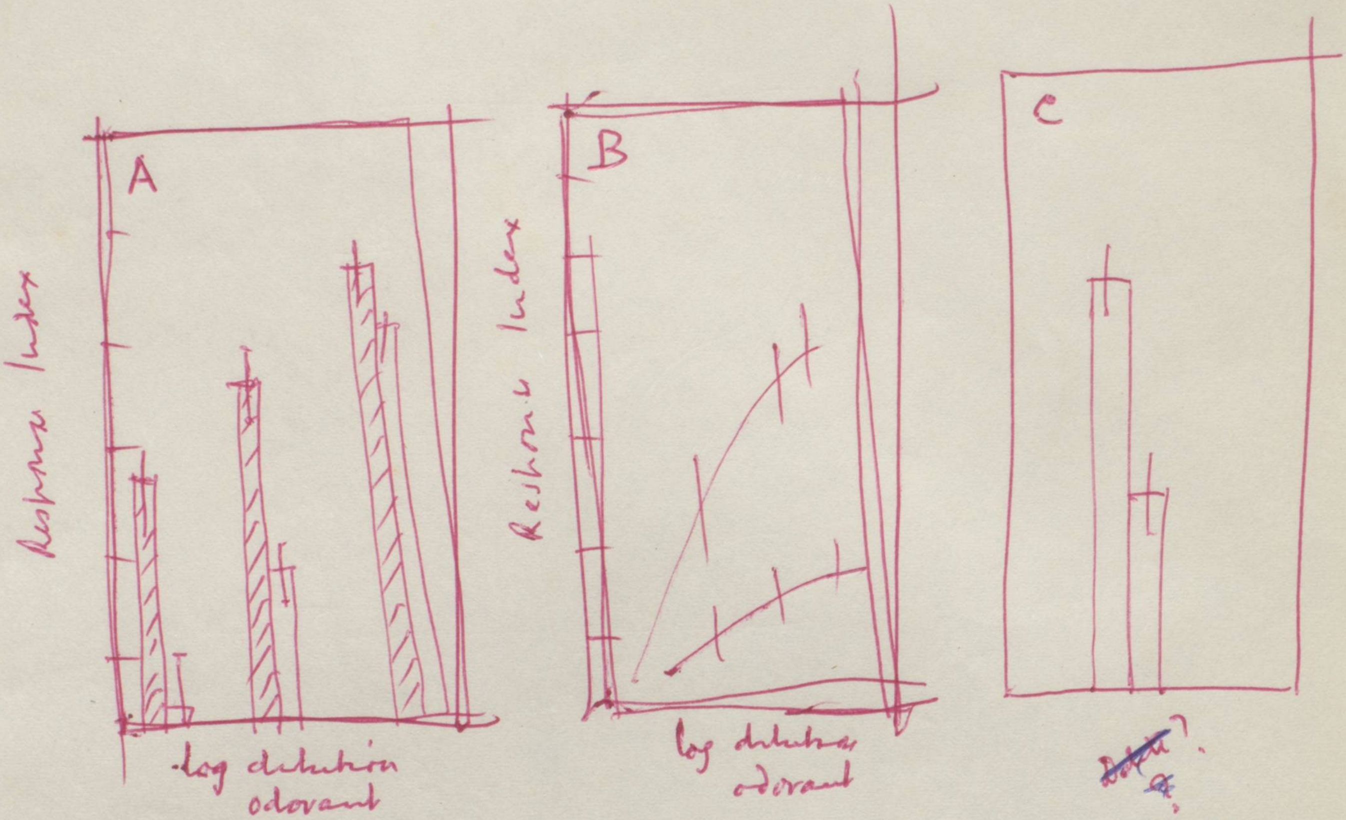
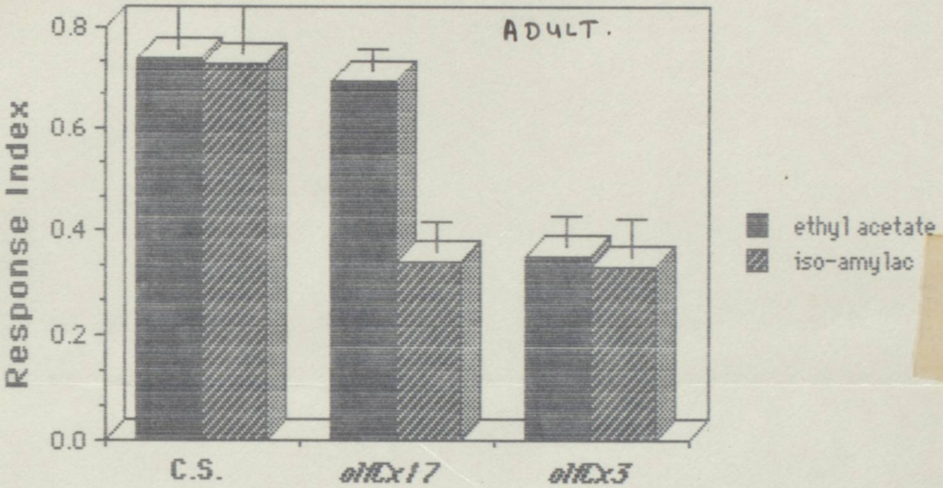


Fig 2. Response of olf A x11 to benzaldehyde: A, response of imago in Y maze; wild type (dark bars); mutant (light bars). B. Larval response; wild type (●); mutant (○). C. Jump response to aldehyde, wild type ( ), olf x11

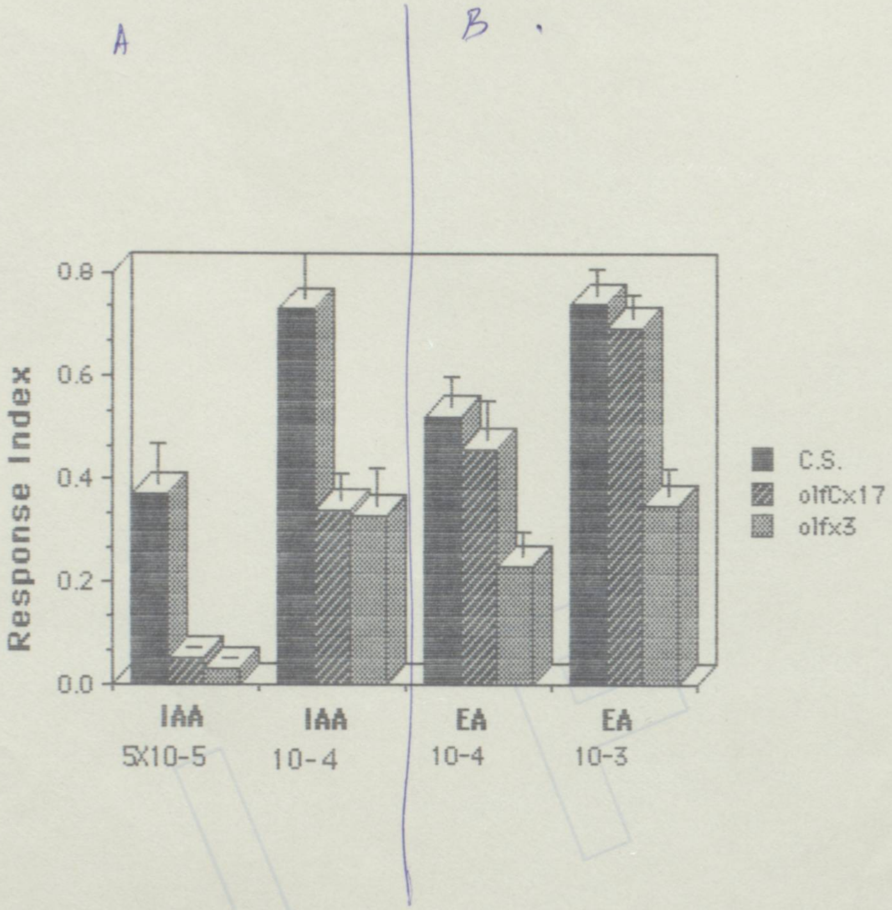


Olf C

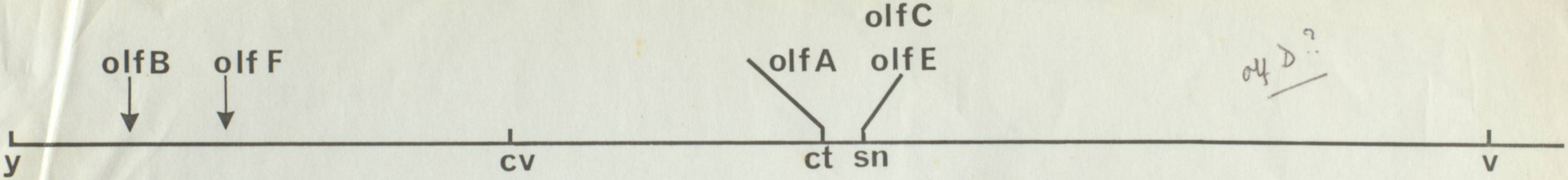
Larval curves to be drawn

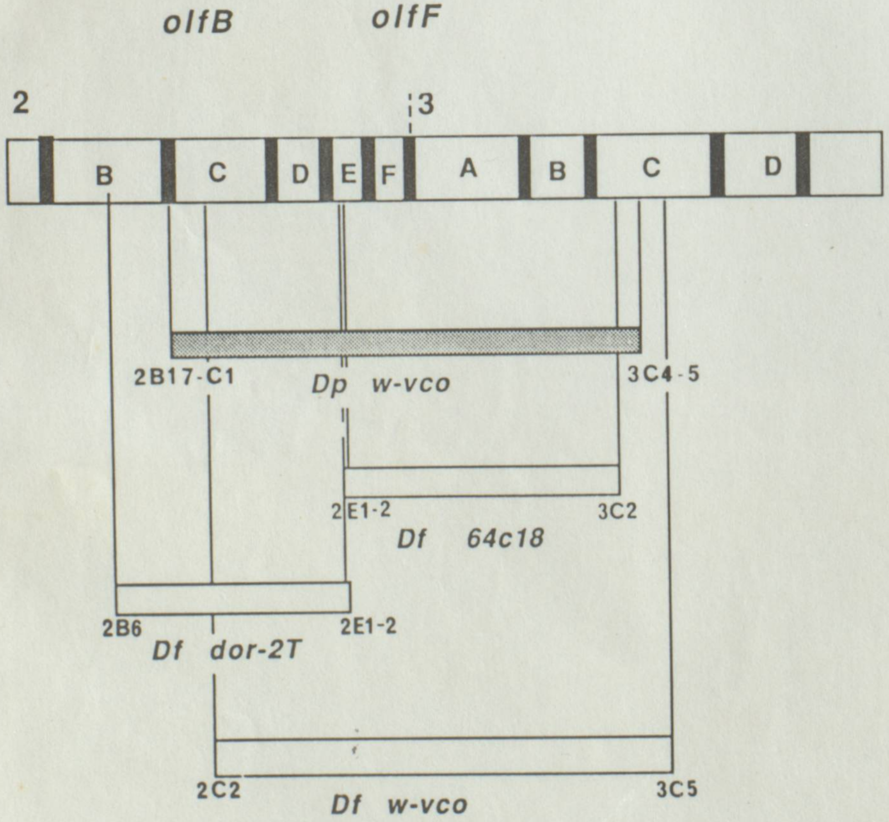
TIFR

Separate



TIFR


olfB
olf F
olfA
olfC
olfE
olf D?
y
cv
ct
sn
v


olfB
olfF
2
3
B
C
D
E
F
A
B
C
D
2B17-C1
Dp w-vco
3C4-5
2E1-2
3C2
Df 64c18
2B6
2E1-2
Df dor-2T
2C2
3C5
Df w-vco

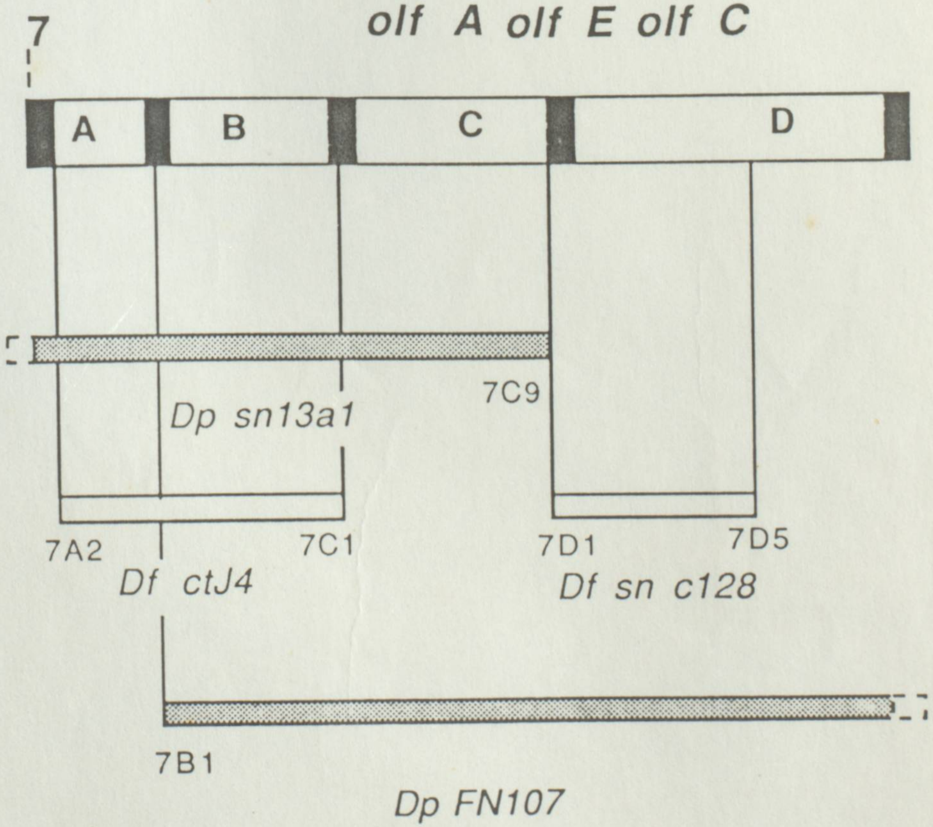
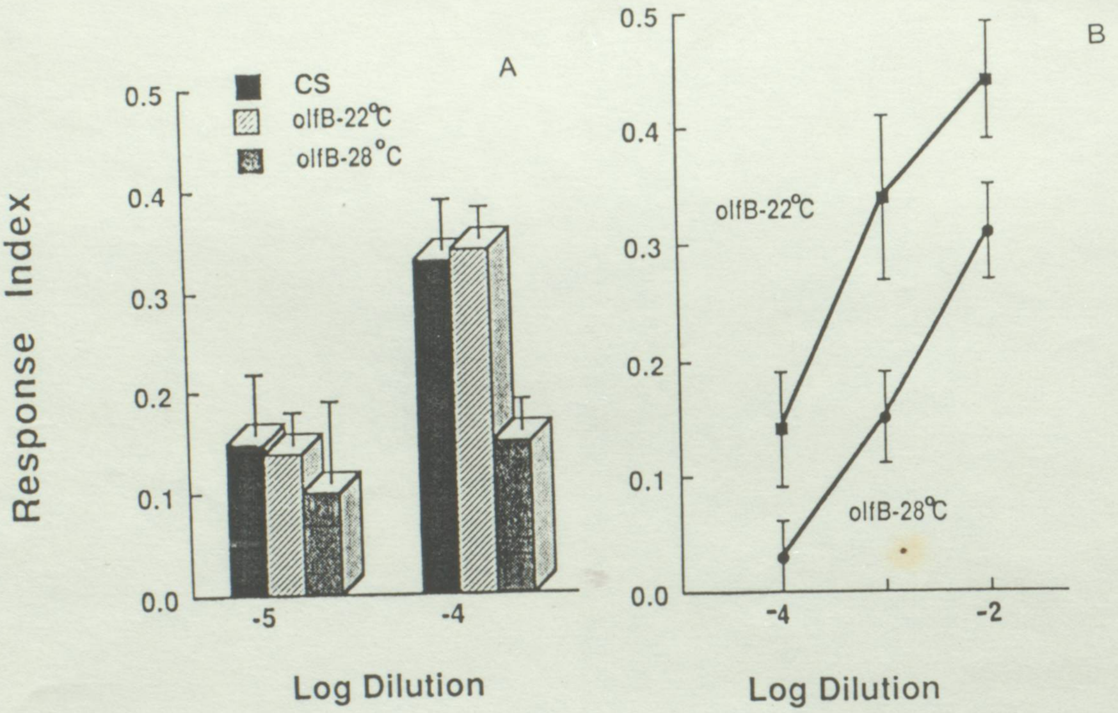

7
olf A olf E olf C
A
B
C
D
7C9
Dp sn13a1
7A2
7C1
7D1
7D5
Df ctJ4
Df sn c128
7B1
Dp FN107

Fig 4



Bars´
[illegible]

Fig 4

Fig 5

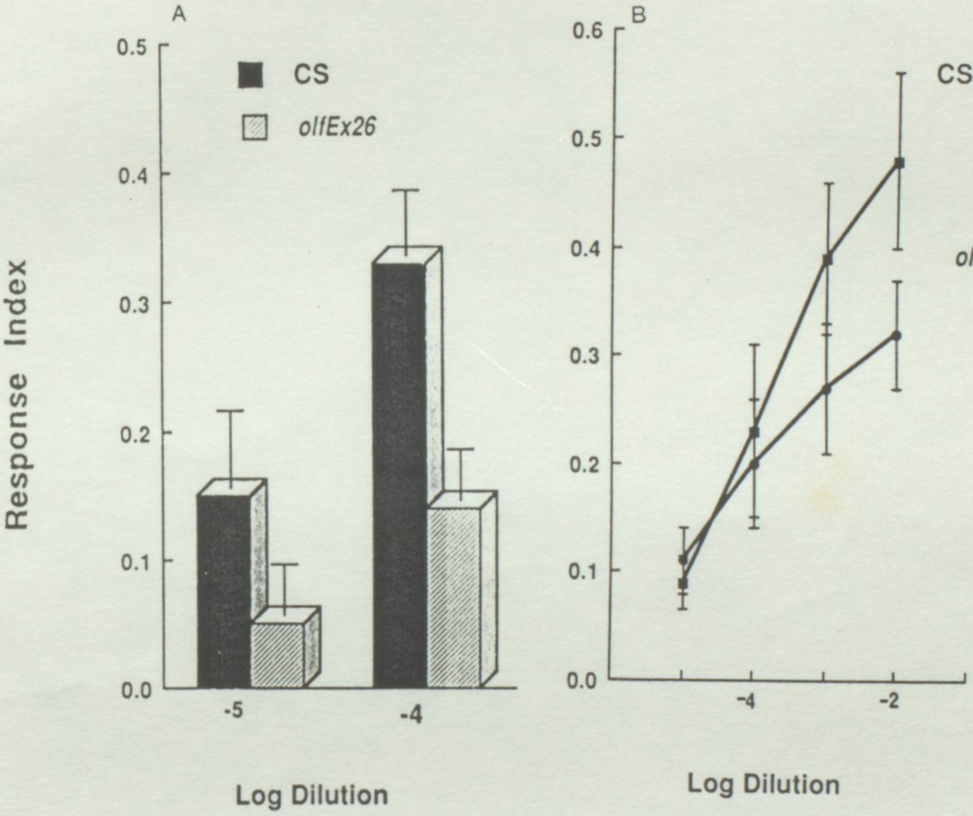
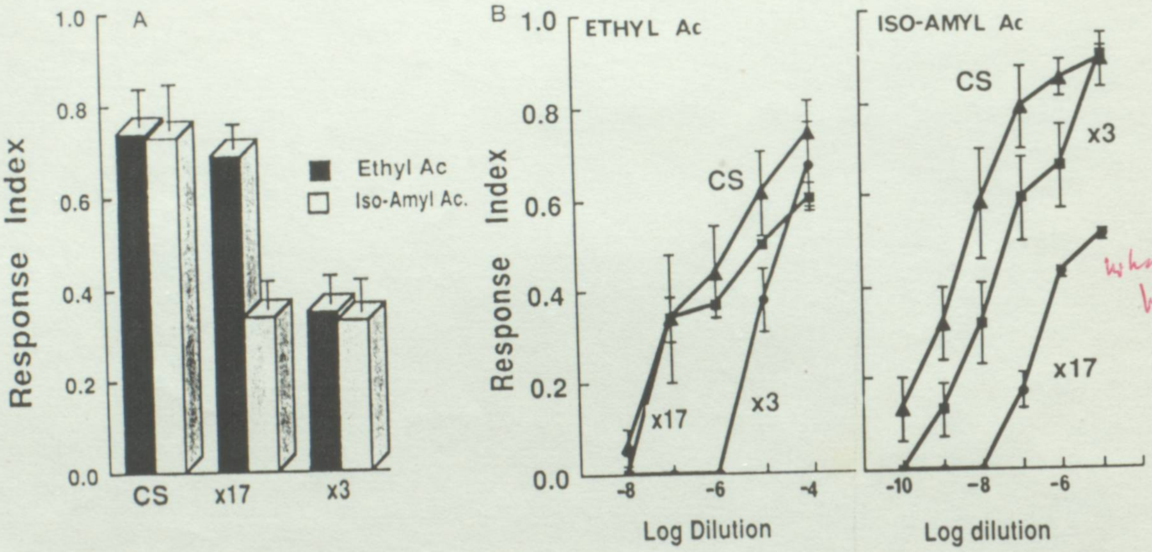

A
0.5
0.4
0.3
0.2
0.1
0.0
Response Index
CS
olfEx26
-5
-4
Log Dilution
B
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CS
olfE
-4
-2
Log Dilution

Fig 5

Fig 6



why no bars here?

Fig 6-

Fig 8

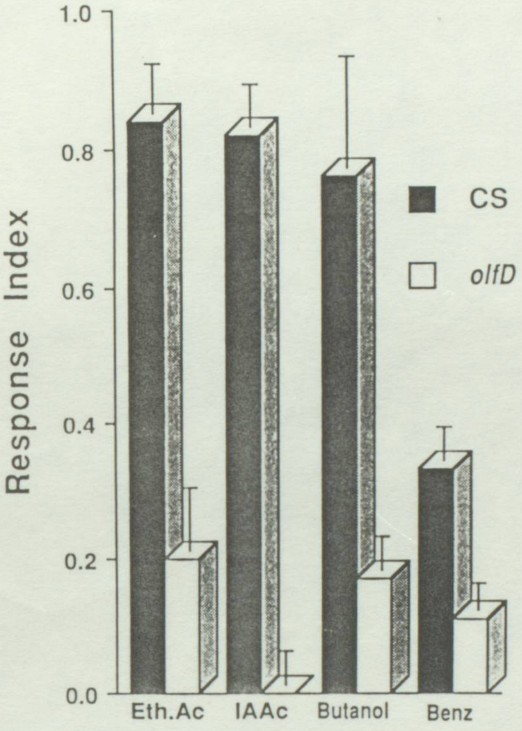
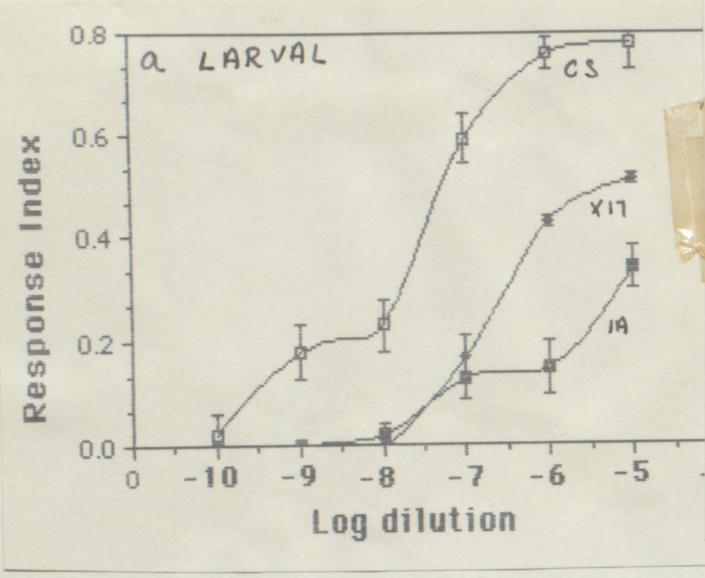
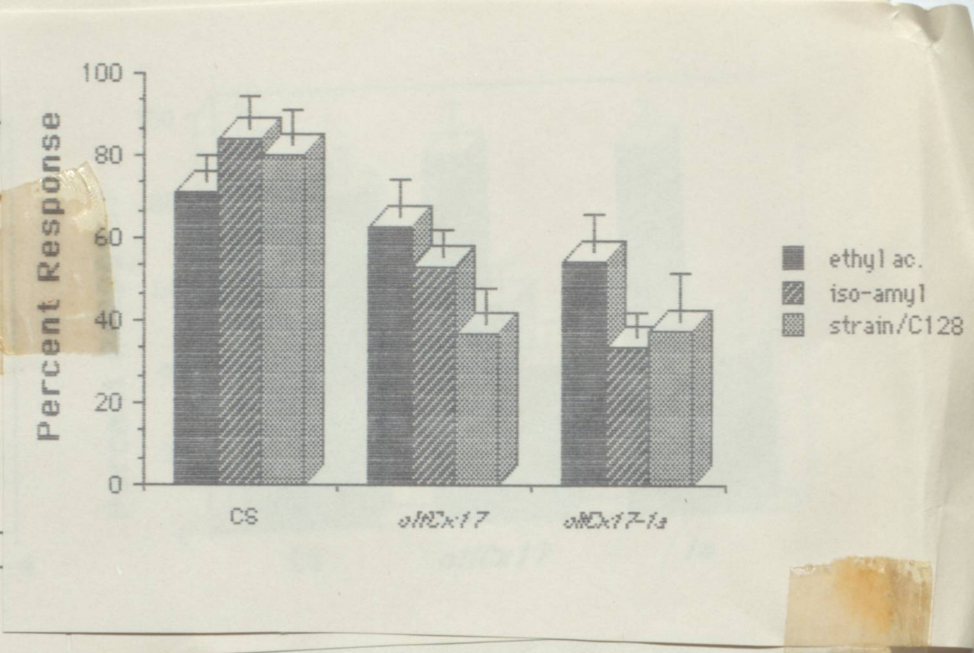


Fig 7

or is it ⑨?

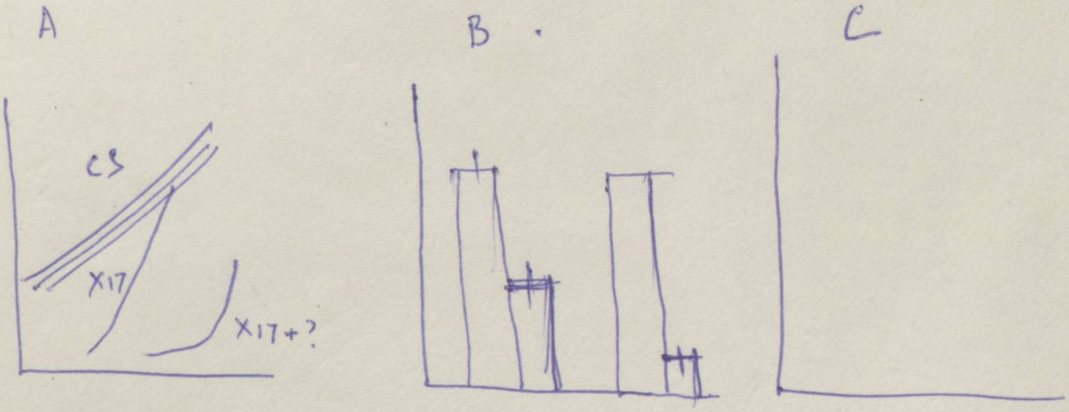
Fig ⑧ summarising the localisation of the *olf* genes. — or should this come earlier?


a LARVAL
CS
X17
1A
Response Index
0.8
0.6
0.4
0.2
0.0
0
-10
-9
-8
-7
-6
-5
Log dilution


Percent Response
100
80
60
40
20
0
CS
olfCx17
olfCx17-1a
ethyl ac.
iso-amyl
strain/C128

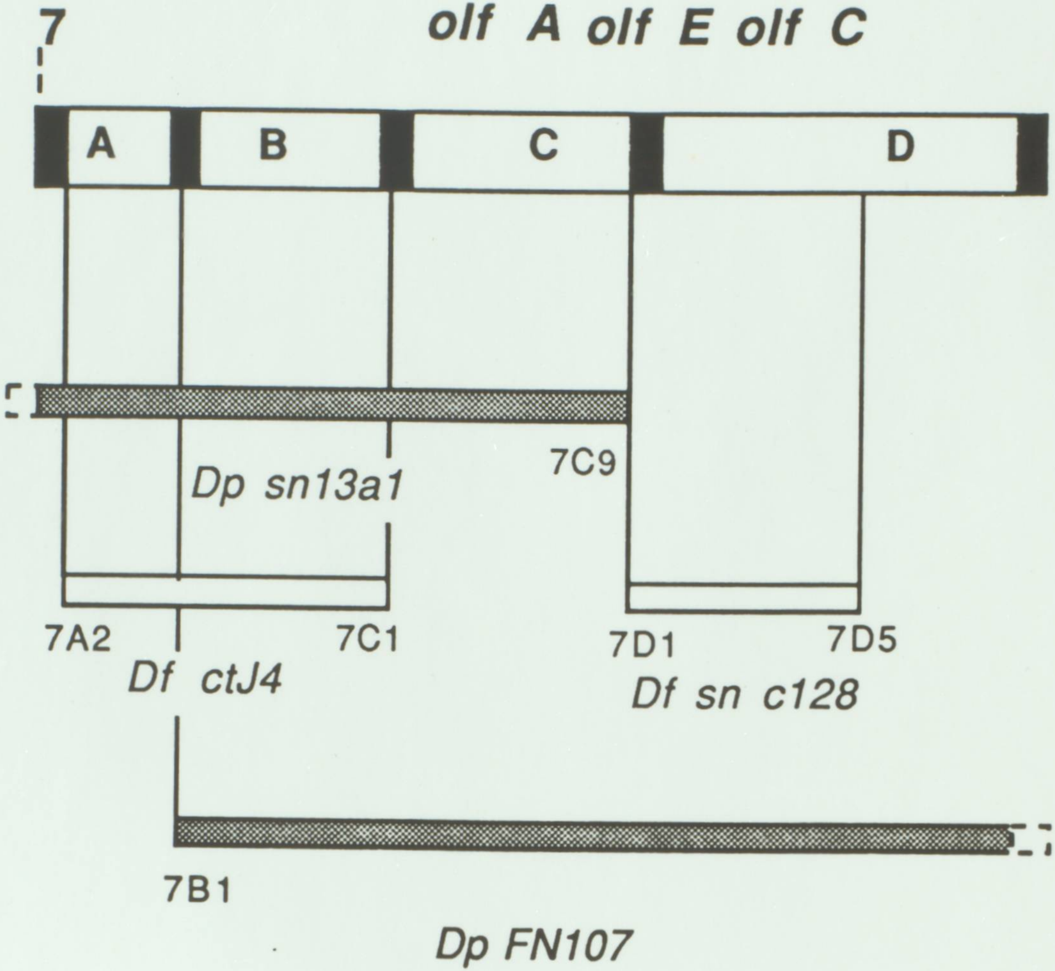
1A

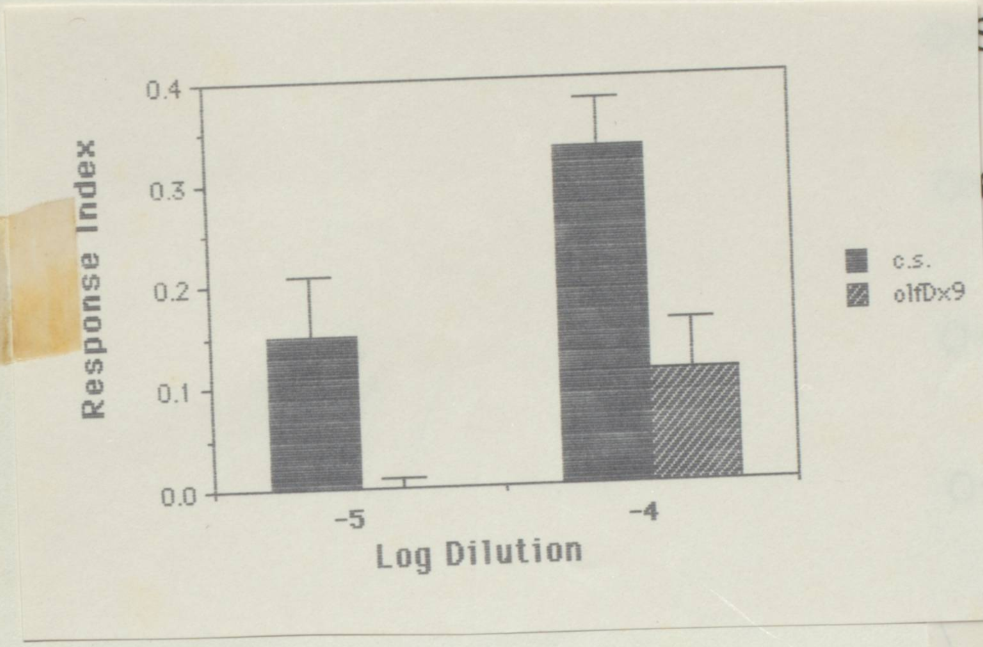
TIFR

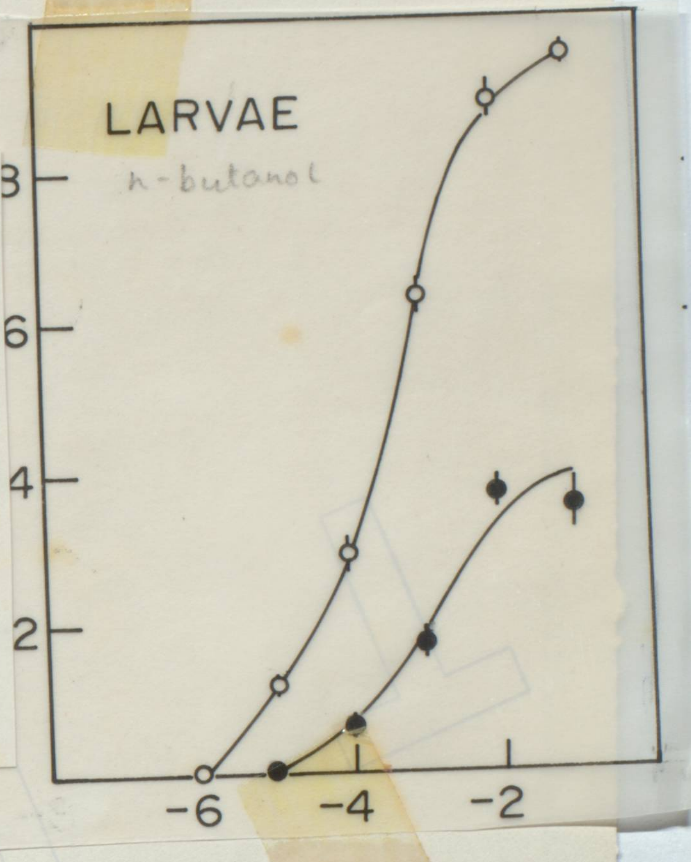


heterozygote
with c128 .

Fig 7. Effect a second step mutation on the responses of olf x17 : A. larval response to iso amyl acetate. B. ~~larval~~ Jump response to ethyl acetate. C. Heterozygote.

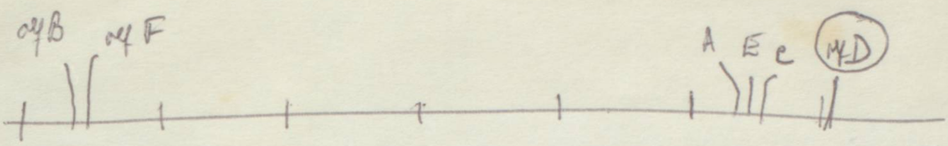

7
olf A olf E olf C
A
B
C
D
7C9
Dp sn13a1
7A2
7C1
7D1
7D5
Df ctJ4
Df sn c128
7B1
Dp FN107


0.4
0.3
0.2
0.1
0.0
Response Index
-5
-4
Log Dilution
■ c.s.
▨ olfDx9


LARVAE
n-butanol
8
6
4
2
-6
-4
-2

respectively. The data suggests that the population d

①



Discussion with Veronica and Gauti.

25/11/90

Cloning.

Transcript.

Breakpoints

alleles.

Existing Genes :-

Alleles - | Isolation of break points

Search for New Genes -

1. Screening of P insert lines

2. Other Approaches :-

X-chromosome

Autosomes

Which chemicals

Biochemistry :-

| Second messenger
| Binding Proteins

Garti.

1. Story of sn mutants
2. Walk
3. Cloning of gust E

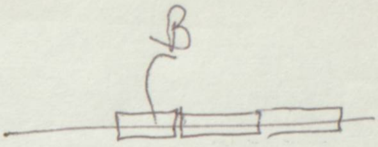
Champa :-

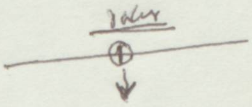
Excision Deficiencies - ?
Which side how large ?

1. Genetics

1. Clone of A and C

Insertion in 7 D1.



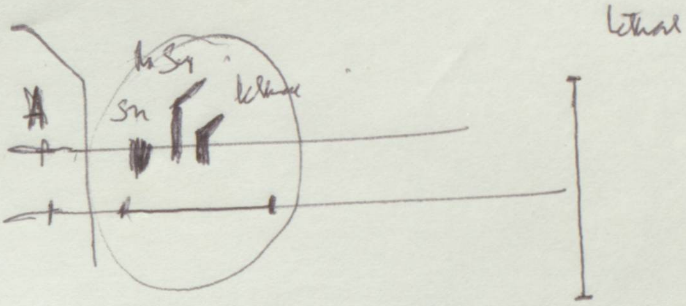


1. Verifying —

X

100g

Chemapra



Results

All dis.

1. Screening :- and testing of deficiencies covering C.

1) Chemapra (Aurnadh)

1. Lethal alleles :-
2. Sn
3. olf

1) Deficiencies
2) Mutations

2) Get more Docs

1) Dominant X Benzaldehyde

Ethyl

?

2) Acetaldehyde + Iso-amyl. (EMS)

× Aroma

40% 40%

Where is it?

3) Aldehyde + Iso-amyl. (P)

29%

?

---

4) Aldehydes (Mutants)

LIST OF OLFACTORY MUTANTS

Genotype	Chemicals tested against Benzaldehyde (10 -4)	Ethyl acetate	Iso amyl acetate (neat J.T.)
Csdg	W.T. -0.37	W.T.	W.T. 90 %
IA	M -0.13	Slightly mutant	M 30 %
RO 27	M -0.11 ✓	W.T.	-
Olf C X17	W.T. -0.34	W.T.	M 40 % ✓
8B	W.T. -0.27	W.T.	M 40 % ✓
Olf E X26	M -0.18	-	-
V - 137	M -0.07	-	M ?
3C/3C	M -0.17	-	
APO 5	M -0.15		
APO 35	M -0.13		
APO 38	M -0.16		
APO 14	M -0.17		
APO 31	M -0.08		
Olf A X1	M -0.17		
Olf C X3	W.T.		M 40 %

maps to y-cv.

multiple P

R 0-27 Data

Chemical used = Benzaldehyde

Conc. used = 10^{-4} & 0.5×10^{-3}

Csdg = $10{-4}$ = 14 readings.
Mean = − 0.37
S.D. = 0.05

0.5×10^{-3} = 8 readings
Mean = − 0.93
S.D. = 0.03

R 0- 27 = 10^{-4} = 16 readings
Mean = − 0.12
S.D. = 0.07

0.5×10^{-3} = 17 readings
Mean = − 0.63
S.D. = 0.15

---

1) Csdg_F X R027_M

Csdg (M) = − 0.33 +/− 0.04
N = 7

Csdg/ R027 (F) = − 0.16 +/− 0.02 Dominant?
N = 7

2) R027_F X Csdg_M

R027 (M) = − 0.12 +/− 0.02
N = 4

R027/ Csdg (F) = − 0.16 +/− 0.02 Dominant?
N = 6

Comparative Data of mutants for Benzaldehyde

Cjump.

1) Csdg = 88.5 % +/- 11.5
N = 10 (20)

2) Olf E v.r. = 33.0 % +/- 10.7
N = 10 (20)

3) Olf E l.p. = 30.5 % +/- 6.1

4) Olf A^{X1} = 47.5 % +/- 10.8
N = 10 (20)

5) V- 137/ V-137 = 20.5 % +/- 11.9 strong
N = 10 (20)

6) Olf E s.j. = 36.5 % +/- 12.5
N = 10 (20)

7) RO27 = 28.5 % +/- 12.5
N = 10 (20)

8) IA = 38.5 % +/- 6.7
N = 10 (20)

9) APO 5 = 29 % +/- 11.3
N = 10 (20)

10) Olf E jmp = 35.5 % +/- 12.8
N = 10 (20)

11) APO 31 = 18.5 % +/- 9.4 strong
N = 10 (20)

Csdg   Df Ctj4   Dp Sn 13 a
----   X   ------- , -----------
Csdg   Ki

f   M

Test used = jump test

Genotype	Iso -Amyl acetate neat	Benzaldehyde neat
a) Csdg , + ----/ --- Ki	88.5 % +/- 5.7 N = 10 (20)	91.5 % +/- 6.7 N = 10 (20)
b) Csdg + ---- , --- CtJ4 Ki	89.0 % +/- 11.3 N = 10 (20)	91.0 % +/- 10.3 N = 10 (20)
c) Csdg Dp ----/, ---- +	88.0 % +/- 9.6 N = 10 (20)	92.0 % +/- 6.9 N = 10 (20)
d) Csdg Dp ---- , ---- Ctj4 +	87.5 % +/- 10.8 N = 10 (20)	89.0 % +/- 7.8 N = 10 (20)

~~to~~ Mapping of olfC with Dp(1;3) sn^{13a1}.

$\frac{\text{Olf C}^{\text{X17}}}{\text{Olf C X17}}$ $\frac{\text{Df CtJ4}}{}$, $\frac{\text{Dp Sn 13 a}}{\text{KI}}$

F M

test used = jump test

Genotype	Iso - amyl acetate neat	Benzaldehyde
a) $\frac{\text{Olf C}}{\text{CtJ4}}$ $\frac{+}{\text{Ki}}$	82.5 % +/- 8.4 N = 10 (20)	80.8 % +/- 7.5
b) $\frac{\text{Olf C}}{\text{CtJ4}}$ $\frac{\text{Dp}}{+}$	82.5 % +/- 9.7 N = 10 (20)	
c) $\frac{\text{Olf C}}{}/$ $\frac{+}{\text{Ki}}$	51.5 % +/- 10.5 N = 10 (20)	90.0 %
d) $\frac{\text{Olf C}}{}/$ $\frac{\text{Dp}}{+}$	49.5 % +/- 11.0 N = 10 (20)	

```
Olf C             Df CtJ4          Dp Sn 13 a
-------     X     -------    ,     ----------
     X3
Olf C                               Ki

test used = jump test

Chemical used = Iso - Amyl acetate (neat )


    Olf C       +
a) ----/ ,    ---          =  47.0 % +/-  8.4
               Ki             N = 10 (20)

    Olf C       +
b) ----- ,    ---          =  80.0 % +/- 13.3
    CtJ4       Ki             N = 10 (20)

    Olf C      Dp
c) -----/,    ---          =  44.0 % +/- 10.3
                +             N = 10 (20)

    Olf C      Dp
d) ----- ,    ---          =  83.5 % +/- 14.1
    CtJ4        +             N = 10 (20)
```

Y - Maze

```
                          -4
Benzaldehyde - 10

Csdg          Df CtJ4     Dp Sn 13 a
----    X     -------  ,  ----------
Csdg                          Ki

F                  M


   Csdg        +
a) ----   ,   ---            =  - 0.36 +/- 0.03
   CtJ4        Ki                  N = 10


   Csdg        +
b) ----/  ,   ---            =  - 0.37 +/- 0.04
               Ki                  N = 10


   Csdg       Dp
c) ----   ,   ---            =  - 0.36 +/- 0.03
   CtJ4        +                   N = 10


   Csdg       Dp
d) ----/  ,   ---            =  - 0.39 +/- 0.04
               +                   N = 10
```

```
Y - maze
                                   -4
Benzaldehyde - 10

IA            Df CtJ4    Dp Sn 13 a
---    X      -------  , -----------
IA                            Ki

F                    M


    IA        +
a)  ---/ ,   ----             =  - 0.13 +/- 0.03
             Ki                     N = 5


    IA        +
b)  ---   ,  ----             =  - 0.13 +/- 0.02
    CtJ4     Ki                     N = 6


    IA        Dp
c)  ---   ,  ----             =  - 0.32 +/- 0.01
    CtJ4      +                     N = 6


    IA        Dp
d)  ---/ ,   ----             =  - 0.35 +/- 0.06
              +                     N = 5
```

Df CtJ4 , Dp Sn 13 a Crosses
Map Position

$\frac{IA}{IA} \times \frac{Df\ CtJ4}{}\ ,\ \frac{Dp\ Sn\ 13\ a}{Ki}$

test used = jump test

Genotype	Iso- amyl acetate neat	Benzaldehyde neat
a) $\frac{IA}{CtJ4}\ ,\ \frac{+}{Ki}$	33.5 % +/- 9.4 N = 10 (20)	21.0 % +/- 9.1 N = 10 (20)
b) $\frac{IA}{CtJ4}\ ,\ \frac{Dp}{+}$	83.5 % +/- 11.5 N = 10 (20)	82.5 % +/- 10.8 N =10 (20)
c) $\frac{IA}{}/\ ,\ \frac{+}{Ki}$	36.5 % +/- 11.5 N = 10 (20)	32.0 % +/- 14.2 N = 10 (20)
d) $\frac{IA}{}/\ ,\ \frac{Dp}{+}$	45.0 % +/- 9.4 N = 10 (20)	75.5 % +/- 11.9 N = 10 (20)

Dear Veronica,

Here are my comments on your manuscript - I give them in three stages. First an overview, dealing with the main issues addressed by the paper. ~~Here I make suggestions~~ Secondly, I ~~will~~ make specific comments ~~these on the script~~, section by section and lastly ~~are~~ editorial remarks written on the manuscript itself.

1 1. Overview:

~~The paper describes the phenotype of the mutants of the loci gustatory genes gust B and gust D and the B strain gust C and Gft and their heterozygotes with the wild type alleles~~ The paper describes 8 gustatory mutants. The key question is, how many loci (cistrons) are involved; where are they located and what can we say about their functions.

Phenotypic groups

The measured properties may be qualitatively summarised as below:

~~Phenotypes~~	~~Gust~~ Attraction by sucrose	Attraction by NaCl	Tolerance to NaCl ~~Repulsion by NaCl~~	Tolerance to Quinine ~~[illegible] against NaCl~~	L1-spike frequency to NaCl	Amplitude of L1 spikes
gust B^{x5}	Normal	Increased,	Increased	Normal	Increased	Bimodal
gust B^{x7}	Normal	Increased	Increased	Normal	Increased	?
gust B^{x7}	Normal	Increased	Increased	Normal	Increased	?
gust C (?)	Reduced	Increased	Increased	Increased	Increased	?
Gft (?)	Reduced	?	?	Increased	Normal (?)	?
gust D^{x3}	?	?	?	increased	Normal	?
gust D^{x6}	?	?	?	Increased	Normal	?

The important question is are we dealing with two or three or four phenotypic groups? This question can be answered with greater confidence if the ~~go empties~~ vacancies (question marks) in the table ~~about~~ ~~can be~~ are filled. ~~One can then decide what symbols to use for~~

~~Let us examine this further~~. The ~~measurement~~ ~~of the~~ phenotypes of the ~~strains~~ mutants and their (dominance test) heterozygotes with ~~their~~ the ~~respective~~ wild type alleles also tells us whether they are similar or different with respect to dominance ~~with respect to dominance~~. I summarise below four results ~~in one table~~ for ease of comparison.

a) Attraction by sucrose (1 mM).

		SE
gust B	Normal	
gust C	40.3	3.8
Gh	13.6	1.4
gust D	?	?
gust C/+	62.3	1.3
Gh/+	64.3	1.3
gust D/+	?	?

gust C and Gh are partially dominant. gust D is ?. It is important to know.

b.) Attraction by NaCl (100 mM).

C.S.	43.5	2.7	
gust B^{x5}	84.9	2.2	
gust B^{x3}	84.9	3.8	(what a coincidence)
gust B^{x4}	70.7	2.9	
gust C	80.8	2.6	
Glt	?		
gust D	?		
gust B^{x5}/+	42.0	1.4	
gust B^{x3}/+	40.3	2.2	
gust B^{x4}/+	39.0	1.5	
Glt /+	?		
gust D/+	?		

Remark: ~~Glt~~ ~~gust~~ gust C is recessive like gust B in this test but what about Glt and gust D? We need to know.

c) Tolerance to NaCl ~~Repulsion by NaCl~~ (0.5 mM)

Data is lacking.

d) Tolerance to Quinine

		SE
CS	4.0	1.2
gust C	43.3	6.1
gust D	38.9	2.5
Glt	45.2	
gust C / +	?	?
gust D / +	24.9	3.5
Glt / +	17.1	2.5

Remark :- gust D and *Glt* are partially dominant. What about *gust C*? We need to know.

e) Firing frequency to NaCl and spike amplitude.

		(SE)	N	Amplitude
CS.	46.2	0.2		Unimodal
gust B^{x5}	60	0.5		Bimodal
gust B^{x7}	56.7	0.5		?
gust B^{xf}	65.4	1.1		?
gust C	59.7	3.1		?
gust D	Normal (2)			?
Gust	Normal (2)			?
gust x^5/+	43.7	1.0		?
gus x^7/+	40.1	1.5		?
gus^{xf}/+	41.1	1.5		?
gust C/+	48.4			

Remark :- gust B firing frequency increase is recessive. Is gust C dominant or recessive? (48.4 spikes) – How about bimodality? We need to know this.

Is the statistical error correctly estimated? (SM or hairs, SM of flies, SM of different experiments).

Summary of remarks ~~etc~~ on phenotypes:

The phenotypes measured in the five types of tests suggest the gust B ~~is a locus~~ and gust D ~~are~~ a distinct locus. ~~gust C and Glt differ in low~~

In NaCl response (spike frequency) gust C is like gust B but in other respects, sucrose response and Quinine-tolerance, it is like gusD and Glt. The gaps in the phenotype measurements need to be filled.

complementation tests

Let us now take a look at grouping by complementation tests.

a.) Sugar attraction

gust D	gust C	Glt	gust H	
?	?	?	?	gust D
	40.3	20.1	?	gust C
		13.6	14.9	Glt
			43	gust H

CS = 89

Remark: Glt and gust C are partially dominant. Double mutant heterozygotes appear to have an extreme phenotype. gust H needs to be separated and described more fully. Complete matrix.

b) Salt attraction

gustB^{x5}	B^{x7}	B^{xf}	gustC	gustD	Glt	
m	m	m	Normal	?	?	Bx^5
	m	m	?	?	?	B^{x7}
		m	?	?	?	B^{xf}
			m			gustC
				m	?	gustD
					?	Glt

Remark :-

x^5, x^7 and x^f belong to a complementation group (ie alleles).

gustC does not belong to this group (is this complementation intergenic or intragenic?)

c) Spike frequency

B^{x5}	~~gustC~~ B^{x7}	B^{xf}	gustC	
60.1 (m)	?	?	66 (m)	x^5
	56 (m)	?	?	x^7
		65 (m)	?	x^f
			59.1	gustC

gust C is apparently allelic to x^5, but what about x^7 and x^f. This does not correlate with the behaviour of the heterozygote. Why?

Are all alleles recessive with respect to firing frequency? (C/+ give 48.4 spikes).

d) Quinine-tolerance.

gust C	gust D^{x3}	gust D^{x6}	Gh	
43	?	?	?	gust C
	38.9	40.1	?	gust D^{x3}
		?	37.8	gust D^{x6}
			45	Gh

Remarks:

All of these mutants are partially dominant (gust C/+ ? ; gust D/+ = 24.9; Gh/+ = 17.1). ~~Table~~ Matrix needs to be completed but there is no evidence of complementation so far. (Tests are incomplete ie C x others) ~~as Dx3 x Bx6 and~~

Summary Remark.

gust B is the only clearly established complementation group so far. with respect to all properties.

Deficiency mapping

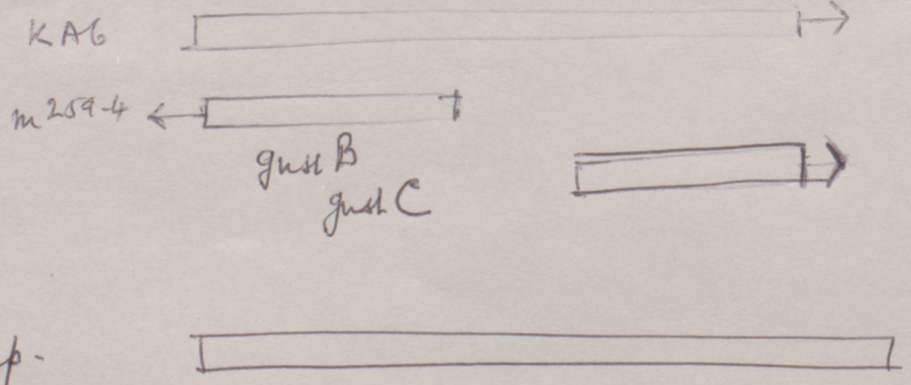
a) gust B and gust C

	Spike frequency to NaCl	(SE)
KA6 / +	38.5	1.7
KA6 / B^{x5}	62.5	1.5
KA^6 / x f	82.2	0.5
$Df(1)m^{259-4}$ / +	48.5	1.3
$Df(1)m^{259-4}$ / B^{x5}	62.2	0.8

~~Spike frequence~~
~~Response~~
~~Alternating Step Shift~~
~~[illegible]~~

$Df(1)m^{259-4}$ / gust C	69.2	1.1
+ / gust C	66.6	2.7
Df m-30 / gust C	?	
Df m-31 / gust B	?	

Result



b) - Deficiency mapping of gust C

	Attraction by sucrose (SE)
C.S	89.0
KA6/+	80.6
Df m^{259-4}/+	87.1
gust C/+	66.6
KA6/gust C	44.8
m^{259-4}/gust C	46.2.

Result is consistent with a).

c) Deficiency mapping of Gil

	Sucrose attraction
KA6/Gil	44.1
m^{259-4}/Gil	87.4
m30/Gil	87.1
v^{L2}/Gil	67
N105/Gil	66
Gil/Gil	13
m30/Gil	?

Remark :- To me these results do not locate Gil unambiguously. Why is it 44 and not about 13?

d) Deficiency mapping of gust D

	Running tolerance
CS	4
D^{x6}/Dup	1.5
gust D^{x6}	38
" D^{x6}/+	24.9
" D^{x6}/KA6	50
" D^{x6}/m^{259}	18
" D^{x6}/m30	41
m30/+	9
m^{259}/+	?
KA6/+	?

Remark

Dx^6/+ is 24.9 (ie D is partially dominant. Then why is D^{x6}/Dup 1.5. I do not understand the variation in tolerances of heterozygotes. Where does gust D fall?

Complementation

②

a) (for sugar attraction)

gust D -	gust C	Gtt	gust H	
?	?	?	?	gust D
	40.3	20.1	?	gust C
		13.6±2	14.9	Gtt
			43.	gust H
				Gus

CS = 89

Gtt and gust C are partially dominant

gust H is recessive (?) Partially dominant

looks like C, Gtt and H are the same – (But H needs to be described more fully).

b) Complementation
(for salt attraction)

gust C	~~gct~~	B^{x5}	B^{x7}	B^{xf}	
m		Normal	?	?	gust C
					~~gct~~
		m	m	m	x^5
			m	m	x^7
				m	x^f.

Result:- x^5, x^7 and x^f are allelic
gust C is not an allele

---

c) Complementation
for timing.

gust C	Bx^5	Bx^7	Bx^f	
59.1	66.	?	?	gust C
	60	?	?	x^5
		56.7	?	x^7
			65	x^f

C/+ 48 ± (Recessive?)
All others are recessive

C seems allelic to x^5 - what about x^7 and x^f.

Does not correlate with Behaviour

d) Complementation for Quinine Tolerance

first C	first Dx^3	D^{x6}	glt	
43	?	?	?	first C
	38.9	40.1		Dx^3
		?	37.8	D^{x6}
			45	glt

Dx^6 = dominant partially

Dx^3 = dominant partially

glt = dominant partially

C = dominant partially

①

1. Test of Dominance (Sugar Attraction).

1. Sugar Attraction	1.0 M suc Success	SD ~~SEM~~	N	Remark
CS	89.0	2.5		
gust B	(Normal)			
gust C	40.3	3.8		
Get 1	13.6	1.4		
gust D	?			
gust C /+	62.3	1.3		Partially Dominant
Get /+	64.3	1.3		Partially, Dominant
gust D /+	?			

---

gust D should be tested.

Test of Dominance (Salt attraction).

	Attraction by NaCl:	SD	Remarks
CS	43.5	2.7	
gust B^{x5}	84.9	2.2	
gust B^{x7}	84.9	3.8	What a coincidence
gust B^{F}	70.7	2.9	
gust C	80.8	2.6	
gust D	?		
GRL	?		
$B^{x5}/+$	42.0	1.4	
$B^{x7}/+$	40.3	2.2	
$B^{F}/+$	39.0	1.5	
gust C /+	42.4	1.6	
GRL /+	?		
gust D /+	?		

1. gust C is recessive in this test.

Test of Dominance

Salt repulsion

Test of Dominance

Quinine Response

	Tolerance 5 mm Quinine		
		SD	N
CS	4.0	1.2	
~~gust B~~	~~?~~		
gust C	43.3	6.1	
gust D?	38.9	2.5	
Glt	45.2		
~~gust B/+~~			
gust C/+	?	?	
gust D/+	24.9	3.5	
Glt /+	17.1	2.5	

Question

gust D and Glt are partially dominant
How about gust C — We must know this.

Tast of Drosophila

Firing Frequency

		to NaCl (?)	SE	N	~~#~~ Spike Morphology
CS		46.2	0.2	350	Unimodal
gust B^{x5}		60	0.5	90	Bimodal
gust B^{x7}		56.7	0.5	40	?
gust B^{xF}		65.4	1.1	48	?
gust C		59.7	3.1	80	?
gust D	(no change ?)	?	?	?	?
Glt	(no change ?)	?	?	?	?
gust $x^5/+$		43.7	1		?
gust $x^7/+$		40.1	1.5		?
gust $x^F/+$		41.1	1.5	.	?
gust C /+		48.4	1		

---

1. Increase in firing frequency is recessive
What about "bimodality"? -
It may explain increased attraction by salt in gust B, (but not increase attraction or genuine tolerance of gust C (Dominant properties)

Deficiency mapping

gust B

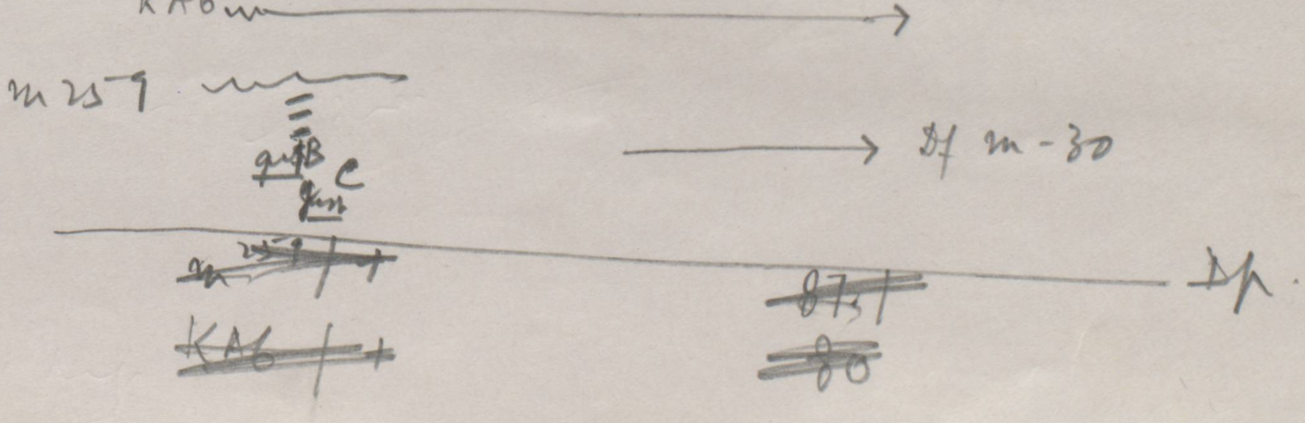
	100 mm NaCl	spike frequency
KA6/+	38.5	1.7
KA6/x^5	62.5	1.5
KA6/x^+	82.2	0.5
Df(1) m^{259}/+	48.5	1.3
Df(1) m^{259}/B^{x5}	62.2	0.8

~~[illegible]~~

~~gust C~~

Df (1) m^{259}/gust C	69.2	1.1
+ / gust C	66.6	2.7
~~KA6 / gust C~~	~~[illegible] 8~~	

Result.



Deficiency mapping

	Sugar response
CS	89.0
KA6/+	80.6
Df m^{259}/+	87.1
gust C/+	66.6
gust C	40.3
KA6/gust C	44.8
m^{259}/gust C	46.2

Deficiency mapping

sel.

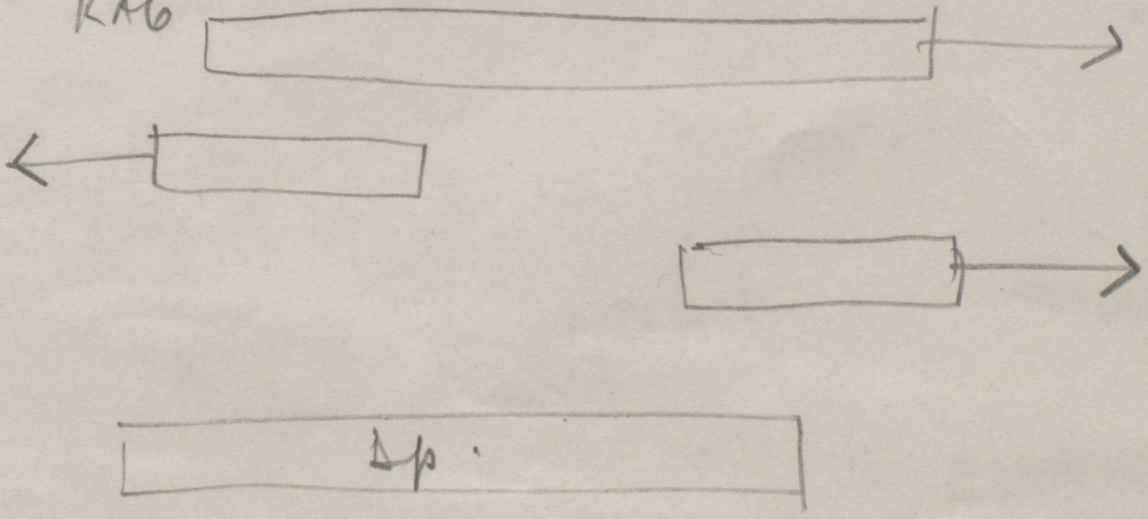
sel / KA6	44.1
gll / $m^{25\text{-}9}$	87.0
gll / m-30	87.1
gll / v^{L2}	67.
gel / N105	66.
gel / 5^{ll}	13.

Inversion–

why is it 44 (not under) KA6

gll is not under any deficiency

Is it under the Duplication?



Deficiency mapping
gust D^-

Quinine Tolerance

CS	④
gust D^{x6}	38
$D^{x6}/+$	24.9
$D^{x6}/KA6$	50
D^{x6}/m^{259}	(18)
D^{x6}/m^{30}	41
D^{x6}/Dp	(1.5)

$m30/+$	9 1.5
$m259/+$	?
$KA6/+$	?

$m30/+$ 91.0